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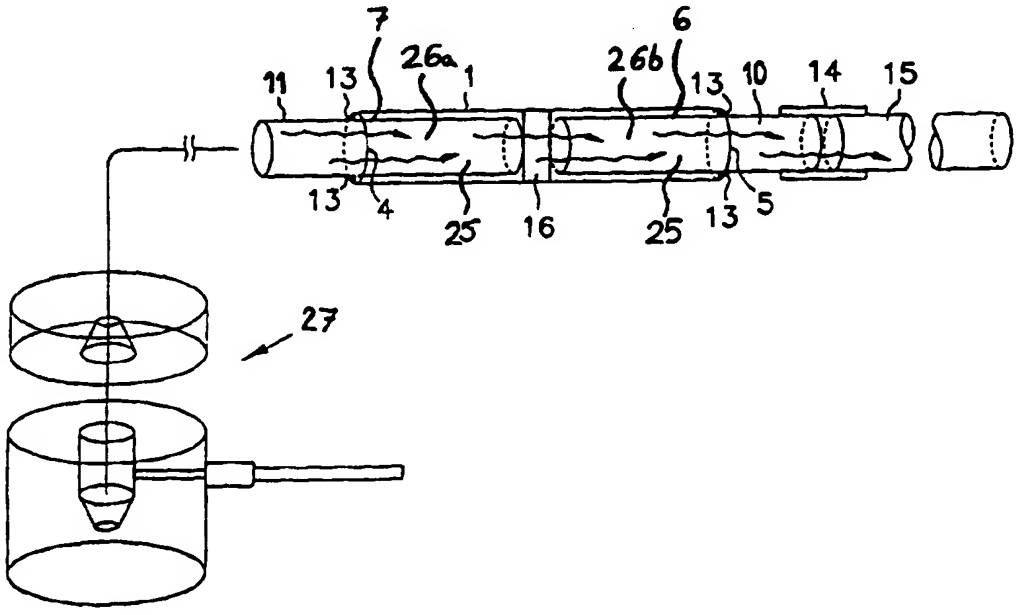

(57) Abstract

A sample preprocessor for use in analytical and preparative chemical analysis is provided. The sample preprocessor contains a sample processing membrane for use in concentrating or chemically processing a sample, or catalyzing a chemical reaction. It is particularly suited to the concentration of dilute samples or the purification of contaminated samples. When used in capillary electrophoresis, the sample preprocessor facilitates reliable and reproducible separation of analytes by eliminating inconsistencies caused by a reversal of the electroosmotic flow otherwise induced by the sample processing material.

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SAMPLE PREPROCESSOR

Background of the Invention

Capillary electrophoresis (CE) is a microscale analytical technique useful for efficiently separating small quantities of different molecular species, known as analytes, present in a chemical or biological sample. A sample containing one or more molecular species is introduced into a separation capillary filled with a suitable electrically conductive buffer. Separation is initiated by placing a large electric potential across the ends of the separation capillary. The resultant electrical field induces species with similar physical and chemical properties to migrate down the separation capillary at similar rates and accumulate into bands, also known as zones. These accumulation bands, also referred to as zones, propagate down the separation capillary and are typically observed by monitoring optical absorption or by using other conventional means of detection.

Capillary electrophoresis can be used to separate analytes in samples that have been subjected to various preprocessing procedures, such as concentration, derivatization, cleavage, oxidation, reduction, or the like. Typically these preprocessing steps are performed before injecting the sample into the capillary electrophoresis system. However, these preprocessing steps often result in sample dilution and necessitate the use of additional manipulations that can contribute to sample loss.

Dilute samples are often of particular interest in clinical, medical, and biological research settings. Often, however, dilute samples cannot often be analyzed effectively because the concentration of analyte within the band of accumulation is below that which is detectable. Thus, sample concentration prior to or during electrophoretic separation or other microscale processing procedures is required.

In addition to suffering from the sample manipulation problems described above, "off-line" methods for sample concentration prior to capillary electrophoresis are exceedingly time-consuming. Thus, in order to enhance the performance of CE,

various on-line analyte electrokinetic focusing techniques have been developed. Electrokinetic techniques generally are those that utilize the induced motion of charged substances subjected to an electrical field. Electrokinetic focusing techniques currently in use include pH dependent sample focusing, isotachophoresis (ITP), and field amplification. However, these techniques can only be applied to charged analytes, and furthermore are of limited use in the analysis of dilute solutions.

As an alternative to focusing and other electrokinetic methods for sample concentration, techniques utilizing on-line adsorbing "pre-columns" have been recently introduced. These techniques utilize a substance at the input end of the separation capillary to capture and concentrate the analyte of interest from a dilute sample. After a sufficient quantity of the analyte has accumulated, the analyte is redissolved in a smaller volume of an appropriate solvent and eluted. Capillary electrophoresis may now be initiated on a concentrated sample, improving the delivery of detectable concentrations of the analytes. These on-line sample concentration techniques may also be used to "clean up" or decontaminate samples prior to electrophoresis. For example, some fluids, such as urine, contain relatively large amounts of salts or other contaminants that need to be removed prior to detecting an analyte that is present in much lower concentrations.

The use of solid phase adsorbing "pre-columns" on a micro- or nano- scale, as in capillary electrophoresis, capillary HPLC, and various mass spectrometry applications utilizing microbore tubing, however, is fraught with difficulty. Increased backpressure caused by the solid phase of the "pre-column" reduces hydrodynamic flow within the capillary and may cause longer migration times and often much broader analyte responses. Additionally, the large volumes of organic solvent often required to elute adsorbed analytes from the solid phase within the capillary may further compromise performance. Peak resolution of the eluted, concentrated analyte deteriorates as the quantity of accumulated analyte increases. Peak broadening is a major drawback of known pre-column concentration methods.

Additional problems result from attempts to use conventional on-line solid phase adsorbing "pre-columns" in

capillary electrophoresis and other micro- or nano-scale applications relying on electrokinetic separation of analytes. Unlike HPLC, which relies on the transfer of analytes from one phase to another to cause separation of analytes, CE relies on placing an electrical potential across the separation capillary to effect separation. Charged analytes and buffer components migrate in response to the resultant electric gradient. The solid phase of the "pre-columns" can act as a physical barrier to this ion flow within the separation capillary, resulting in reduced or anomalous electroosmotic flow (EOF) and nonreproducible analyte migration times. Component resolution can be affected; analyte peaks are broader, and substantial component tailing may occur. Also, incomplete removal of analytes from previous separations can build up on the surface of the solid phase and impair ion flow. Finally, the large volume of organic solvent often needed to elute analytes from the solid phase appears to considerably reduce the EOF. When large volumes of organic solvents are used EOF can also be erratic, leading to peak broadening effects. Bands cannot be compressed without limit, and in very dilute solutions analytes cannot be detected even when employing these more sophisticated focusing techniques.

Where pre-columns are used, separation effectiveness of the capillary electrophoresis system is also reduced because the coatings and solid phase packing materials used to concentrate the sample on-line prior to separation strongly perturb the electroosmotic flow in the separation capillary. A similar perturbation in electroosmotic flow results from coatings and packing materials used to preprocess the sample on-line in other ways, such as by derivatization, reduction, or cleavage. The EOF is the force applied to the analytes as a consequence of the surface charge in the separation capillary induced by the electric field. A reversal in EOF can reduce the efficacy of the separation by reducing resolution, change analyte migration times, or cause some species to travel in the opposite direction, eventually causing their ejection from the inlet side of the separation capillary.

The numerous problems associated with known off-line and on-line methods of sample concentration and other sample preprocessing methods thus severely compromise micro- and nano-scale analytical techniques such as capillary electrophoresis, rendering

them unreliable and irreproducible, hence unsuitable for analytical applications. There exists, therefore, a clear need in the field of micro- and nano- scale bioanalytical chemistry for a method to effectively concentrate or otherwise preprocess components of interest in a dilute sample prior to capillary electrophoretic separation or other analyte processing or detection procedure, in a way that improves reproducibility of results and minimizes sample loss due to handling.

Summary of the Invention

The present invention provides an apparatus useful for preprocessing samples containing analytes of interest prior to analyte processing, separation, detection or analysis. The apparatus, described most generally as a sample preprocessor, is useful for sample concentration, decontamination, and/or preprocessing and can be used either off-line or on-line with a suitable system or device for processing, separating, detecting or analyzing analytes. Suitable systems or devices include, for example, capillary electrophoresis systems, high performance liquid chromatography (HPLC) systems, micro-bore or capillary HPLC, capillary electrochromatography (CEC) systems, mass spectrometers of all types, such as electrospray ionization-mass spectrometers (ESI-MS), microspray or nanospray ESI-MS, and matrix assisted laser desorption time of flight mass spectrometers (MALDI-TOF-MS), and microdialysis. The invention also relates to methods of using the sample preprocessor.

The present invention preferably provides an apparatus and method for performing preconcentration capillary electrophoresis (PC-CE). In particular, this invention relates to sample preconcentration, decontamination, and/or preprocessing prior to, or during, capillary electrophoretic separation of analytes.

Specifically, the invention provides a sample preprocessor comprising a container having a first port, a second port, and an interior space partitioned by at least one membrane into a first compartment and a second compartment. The first compartment comprises the first port, and the second compartment comprises the second port, so that a liquid that enters the interior space through the first port and exits the interior space through the second port traverses the membrane.

The membrane comprises a sample processing material useful for concentrating, decontaminating, or chemically preprocessing components of a liquid sample, and is disposed inside the container so that each of the sample components flowing through the container traverses the membrane and thereby physically contacts the sample processing material. In capillary electrophoresis applications, the use of a membrane is believed to effectively surmount the electroosmotic flow (EOF) reversal problem associated with conventional pre-column concentrators, such as those containing solid phase particulate packing material, and other analyte concentration and preprocessing techniques used in the art.

Also provided by the invention is sample preprocessor assembly, which comprises a sample preprocessor, means for introducing a liquid sample into the first port via an inlet connection, and means removing and recovering a preprocessed sample from the second port via an outlet connection, such that the sample introduction means is in fluid communication with the first port, and the sample removal means in is fluid communication with the second port. The sample preprocessor assembly further includes a sealant for sealing the inlet and outlet connections so as to prevent the leakage of liquid from the connections.

The preprocessor assembly can be placed in-line with a suitable system or device for processing, separating, detecting or analyzing analytes, by using a connection means to place the sample removal means in fluid communication with a capillary useful for electrophoretic or chromatographic separation, or delivery to the desired processing system or detection device. In a preferred embodiment for use in capillary electrophoresis, the invention provides a sample preprocessor assembly that can be connected to an existing capillary electrophoresis system by using a connector to place the sample removal means, typically a microbore capillary electrophoresis tube, in fluid communication with a separation capillary.

The invention also provides a sample preprocessing system comprising at least one sample preprocessor assembly in fluid communication with the inlet end of a capillary. In a preferred embodiment, the sample preprocessing system further includes one or more suitable systems or devices for processing, separating,

detecting or analyzing preprocessed analytes. In an embodiment for use in capillary electrophoresis, the invention provides a preprocessing capillary electrophoresis system, wherein the sample removal means of at least one sample preprocessor assembly is in fluid communication with the inlet end of a separation capillary.

The invention further provides method for off-line use of the sample preprocessor wherein a liquid sample containing a plurality of analytes is brought into contact with the sample processing membrane of a sample preprocessor to yield at least one preprocessed analyte. In a preferred method, the sample preprocessor contains an adsorptive membrane, and the analyte adsorbs onto the membrane so that it is extracted from the carrier medium and immobilized and concentrated on the membrane. The concentrated analyte can be eluted from the container by supplying an eluent effective to remove the analyte from the membrane. After elution, the preprocessed analyte can be detected or further processed or analyzed, as desired. Alternatively, the immobilized, concentrated analyte can be further processed while immobilized on the adsorptive membrane, by contacting the immobilized analyte with various reagents such as derivatizing agents or the like, prior to elution.

A method for preprocessing samples on-line prior to subjecting the preprocessed analytes to other analytical, processing, separation or detection methods is also provided. A liquid sample containing a plurality of analytes is brought into contact with the sample processing membrane of a sample preprocessor of a sample preprocessing system to yield at least one preprocessed analyte. The preprocessed analyte is then moved from the sample preprocessor into a capillary, after which it is processed, separated, detected and/or analyzed.

In a preferred method, known as "preprocessing capillary electrophoresis", the liquid sample is brought into contact with the sample processing membrane present in the sample preprocessor of a preprocessing capillary electrophoresis system to yield at least one preprocessed analyte, and the preprocessed analyte is separated from other sample components or analytes by introducing at least one electrically conductive buffer into the preprocessing capillary electrophoresis system and applying an electric field, e.g., of about 1-60 kV across the preprocessing capillary electrophoresis

system to yield a separated analyte. The separated analyte is then focused and detected.

The method for performing preprocessing capillary electrophoresis uses an analyte focusing technique to improve resolution of an electrophoretically separated analyte. Transient isotachophoresis is a preferred analyte focusing technique. In a particularly preferred method involving preconcentration of at least one analyte, the preprocessing capillary electrophoresis system is filled with an electrically conductive buffer, and a liquid sample containing at a plurality of analytes is introduced into the sample preprocessor so as to contact and adhere to a sample processing membrane containing an adsorptive material for concentrating an analyte. Next, a small volume of aqueous alkaline solution, referred to herein as an alkaline plug, is introduced into the first port of the container. At least one concentrated analyte is then eluted from the container by supplying one or more elution buffers that release the analyte from the adsorptive membrane, and immediately thereafter a small volume of aqueous acid solution, referred to herein as an acid plug, is introduced into the first port of the container. The concentrated analyte is then separated from other sample components or analytes by introducing an electrically conductive buffer into the first port of the container and applying an electric field of, e.g., about 1-60 kV across the preprocessing capillary electrophoresis system. The separated analytes are detected using any conventional detection method known in the art.

Preconcentration capillary electrophoresis can be performed on multiple samples using a plurality of sample preprocessors. In a preferred embodiment, preloaded sample preprocessors containing concentrated, immobilized analytes are used individually in succession in a capillary electrophoresis system to substantially increase the rate of analysis. Multiple preloaded sample preprocessors can be prepared in advance. If desired, the immobilized, concentrated analytes present in the preloaded sample preprocessors can be further processed while immobilized on the adsorptive membrane, by contacting the immobilized analyte with various derivatizing agents or the like. A preloaded sample preprocessor is then connected to a capillary electrophoresis system, and the concentrated immobilized analyte is eluted,

electrophoretically separated, and detected. The sample preprocessor is then removed from the system and a second preloaded sample preprocessor is connected. A regenerative step is performed on the capillary electrophoresis system, if needed. The process is repeated for all preloaded sample preprocessors.

Alternatively, instead of being prepared in advance, preloaded sample preprocessors can be prepared while a capillary electrophoresis experiment is in progress, and the sample preprocessors used in the experiment can be rotated on and off the capillary electrophoresis system. Thus, the invention provides a method for performing preconcentration capillary electrophoresis of multiple samples preconcentrated off-line comprising:

(a) contacting a liquid sample comprising a plurality of analytes with the at least one membrane of a first sample preprocessor assembly, wherein the at least one membrane comprises an adsorptive material for concentrating an analyte, such that at least one analyte adsorbs onto the membrane to yield a first concentrated analyte;

(b) connecting sample removal means of the first sample preprocessor assembly to the inlet end of a separation capillary of a capillary electrophoresis system;

(c) separating the first concentrated analyte by introducing at least one electrically conductive buffer into the capillary electrophoresis system and applying an electric field of, e.g., about 1-60 kV across the capillary electrophoresis system to yield a first separated analyte;

(d) concurrently with step (c), contacting a liquid sample comprising a plurality of analytes with the at least one membrane of a second sample preprocessor assembly, wherein the at least one membrane comprises an adsorptive material for concentrating an analyte, such that at least one analyte adsorbs onto the membrane to yield a second concentrated analyte;

(e) detecting the first separated analyte;

(f) disconnecting the sample removal means of the first sample preprocessor assembly from the inlet end of the separation capillary;

(g) connecting sample removal means of the second sample preprocessor assembly to the inlet end of the separation capillary of the capillary electrophoresis system;

(h) separating the second concentrated analyte by introducing at least one electrically conductive buffer into the capillary electrophoresis system and applying an electric field across the capillary electrophoresis system to yield a second separated analyte;

(i) concurrent with step (h), repeating step (a);

(j) detecting the second separated analyte; and

(k) repeating steps (b) through (j).

In a preferred method for preconcentration capillary electrophoresis of multiple samples, samples are eluted from the membrane with an effective elution buffer prior to the electrophoretic separation. A first elution step is performed immediately preceding step (c), wherein the first concentrated analyte is eluted from the container of the first sample preprocessor assembly by supplying at least one elution buffer that releases the first concentrated analyte from the adsorptive material, and a second elution step is performed immediately preceding step (h), wherein the second concentrated analyte is eluted from the container of the second sample preprocessor assembly by supplying at least one elution buffer that releases the second concentrated analyte from the adsorptive material.

More preferably, the method for analyzing multiple samples includes analyte focusing steps prior to detection of the separated analyte. A first analyte focusing step is performed immediately preceding step (e) to focus the first separated analyte, and a second focusing step is performed immediately preceding step (j) to focus the second separated analyte.

The invention can be employed by disciplines which include, but are not limited to, clinical diagnosis, forensic sciences, environmental chemistry, agrochemical analysis, petrochemical analysis, food chemistry, and other branches of analytical science and research. The present invention is particularly useful for clinical diagnosis because it permits more rapid and accurate analyses of extremely small quantities of drug metabolites, peptides, and proteins from biological or physiological fluids such as urine, feces, serum, blood, plasma, cerebral spinal fluid, nasal drainage, lymph fluid,

aqueous humor, various biopsies, tissue homogenates, cultured cells, and/or extracts thereof.

Description of the Figures

Figure 1 shows a magnified view of a sample preprocessor assembly, wherein adsorptive particles commonly used in high performance liquid chromatography (HPLC) packings constitute the sample processing material.

Figure 2 shows a magnified view of a sample preprocessor assembly containing a membrane that can be used in analyte extraction.

Figure 3 shows a magnified view of a sample preprocessor assembly containing two types of adsorptive membranes, typically chemically derivatized membranes.

Figure 4 shows a magnified view of the sample preprocessor assembly of Figure 1 with a connector that includes two pieces of organic polymer tubing.

Figure 5 shows an ion chromatogram of each sample component as well as a total ion current chromatogram for the CE experiment described in Example 3, obtained using a sample preprocessor containing adsorptive particulate material.

Figure 6 shows an ion chromatogram of each sample component as well as a total ion current chromatogram for the CE experiment described in Example 3, obtained using a sample preprocessor containing adsorptive particulate material, and following the preseparation concentration with transient isotachophoresis to improve analyte resolution.

Figure 7 shows an ion chromatogram of each sample component as well as a total ion current chromatogram for the CE experiment described in Example 4, obtained using a sample preprocessor containing adsorptive particulate material, and

following the preseparation concentration with field amplification to improve analyte resolution.

Figure 8 shows an ion chromatogram of each analyte obtained using a sample preprocessor containing an adsorptive membrane, and following the preseparation concentration with transient isotachophoresis to improve analyte resolution.

Figure 9 shows an ion chromatogram of each analyte component of peptide mixture, obtained using a sample preprocessor containing an adsorptive membrane, and following the preseparation concentration with transient isotachophoresis using larger volumes of leading, trailing and elution buffers to improve analyte resolution.

Figure 10 shows an ion chromatogram of each of the various drug metabolites of halperol found in an unprocessed urine sample, obtained using a sample preprocessor containing adsorptive material.

Figure 11 shows a magnified view of a sample preprocessor containing a membrane that can be used in analyte extraction.

Figure 12 shows a magnified view of a sample preprocessing system, wherein pressurized nitrogen gas is used to move a liquid sample through the container so as to contact an adsorptive membrane, and wherein the second port is connected by a capillary to an electrospray ionization-mass spectrometer detection device.

Figure 13 shows a magnified view of two sample preprocessors used in a single preprocessing capillary electrophoresis system.

Figure 14 shows an ion chromatogram of protein components of an aqueous humor sample, obtained using a sample preprocessor containing an adsorptive membrane, in an experiment utilizing matrix-assisted laser desorption time of flight mass spectrometry.

Detailed Description of the Invention

In capillary electrophoresis (CE), a separation capillary typically made from a length of microbore capillary electrophoresis tubing (which typically has an internal diameter of about 20-200 μm), is filled with an electrically conductive buffer. A sample of interest, typically containing a multiplicity of molecular species referred to herein as analytes, is then introduced into the separation capillary. The pH and ionic strength of the electrically conductive buffer are generally chosen to maximize the differences in the ionization states of the various molecular species present in the sample. Separation is initiated by placing an electric potential (typically about 1-60 kV) across the ends of the separation capillary.

The applied electric field generates a two component force that acts on the molecular species present in the injected sample. First, an attractive or repulsive electrostatic force, proportional to the mass to charge ratio of the analyte, is applied directly to each ionized species in the sample. This is sometimes referred to as the electrophoretic force. Second, the electrolyte in the buffer experiences a force due to the applied electric field as a consequence of the surface charge in the separation capillary. This second force is called the electroosmotic or endoosmotic flow (EOF), and is directly proportional to the gradient of the applied electric field. The electroosmotic flow occurs because ions located in the double layer present at the capillary/liquid interface move under the force of the applied electric field, and the flow of these ions hydrodynamically drives the flow of the bulk electrolyte within the separation capillary.

The action of the resultant force on the molecular species induces their migration down the narrow diameter capillary tubing. The combined applied force induces species with similar physical and chemical properties to migrate at similar rates and accumulate into bands or zones. The continued application of the electric potential on the ends of the separation capillary causes these bands to propagate down the capillary. An optical detector, is typically employed at, or near, the output end of the separation capillary in order to observe these bands of analytes. The process variables, including, for example, pH, electrolyte species, ionic strength, organic

content of run buffer, sample preparation, and loading buffer, temperature, voltage, and the like, can be adjusted by those skilled in the art in order to achieve effective separation.

Samples are commonly subjected to enzymatic or nonenzymatic chemical preprocessing prior to electrophoresis. For example, the samples can be radioactively or fluorescently labeled in order to make additional detection options available. Another example is the facilitation of protein sequencing by subjecting a purified protein sample to enzymatic digestion to generate peptide fragments that can be separated electrophoretically. Such preseparation processing steps, however, contribute to sample loss and dilution.

Dilute samples are difficult to analyze when the concentration of analyte within the band of accumulation is below that which is detectable using standard optical techniques. One method of improving the detection of dilute analytes is to use more sensitive detection methods. For example, the mass spectrometer, a very sensitive and versatile detector, has been employed in conjunction with capillary electrophoresis. The use of a mass spectrometer as a detector is attractive because the detection limits of CE can be extended to low analyte concentrations where optical detection is not effective. Further, mass spectrometry measures charge-to-mass ratio, yielding information about molecular weight, which is more useful for the direct identification of an analyte than is optical absorbance. It is therefore possible to detect multiple species present in analyte bands produced by CE. Structural information about the analyte can also be acquired using the fragmentation techniques routinely employed by those skilled in the art of mass spectrometry. Nonetheless, in spite of the improved sensitivity and more complete chemical information provided by mass spectrometric detection, the analysis of extremely dilute samples by capillary electrophoresis remains challenging.

Many other sensitive and useful detection techniques are known in the art. The technique chosen typically depends on the nature of the analyte. Examples of other useful detection methods are based on optical fluorescence, electrochemical oxidation or reduction, plasma resonance, radioactivity, refractive index, and conductivity.

Very dilute analytes can remain undetectable despite the use of the most sensitive of known detection methods.

Another important way to improve detection of dilute analytes, therefore, is to concentrate them prior to, or concurrent with, separation. Preseparation or concurrent analyte concentration, coupled with the use of a sensitive detection method, greatly increases the usefulness and efficacy of CE. Known off-line preseparation concentration methods, however, are time-consuming and suffer from various sample-handling risks such as contamination or sample loss due to spill or adsorption onto container walls.

As a result, various on-line focusing methods have been developed in response to these problems. Field amplification (also known as sample stacking, analyte stacking, or moving boundary stacking) is one of the simplest methods for on-line sample concentration. The ionic strength differences between the sample matrix and the separation buffer are exploited. The separation capillary is filled with an electrically conductive separation buffer. A small volume of sample, commonly referred to as a sample plug, containing a low conductivity buffer is then introduced into the separation capillary, followed by additional separation buffer. Because charged analytes have an enhanced electrophoretic mobility at lower conductivity, when voltage is applied, the electric field is amplified and the analytes quickly accelerate toward an interface, at which they are concentrated. In the low conductivity region, the electroosmotic flow is reduced, and analyte movement is generated primarily by electrophoretic mobility at the sample pH. Generation of excess heat in the sample plug is a problem with this technique, and to avoid the heat-induced denaturation of sample components, analytes may be "stacked" at low voltage and then separated at higher voltage.

Field amplification can be increased if a small volume of water is introduced, followed by the sample of interest, and then by an acidic stacking buffer. The water acts as a temporary barrier to ion current flow which results in the local amplification of the electric field gradient adjacent to the elution media (R.-L. Chien et al., J. Chromatog., 559, 141-152 (1991); R.J. Nelson et al., Handbook of Capillary Electrophoresis, J.P. Lander, ed., CRC Press, Boca Raton, FL (1994)). This local change in the electric field gradient provides a

barrier against which the analyte zone can become compressed or focused in space by the flow of ionic species from the acidic stacking buffer.

Another class of focusing methods useful for sample concentration is pH-dependent sample focusing, wherein a high pH sample plug is flanked between low pH separation buffer zones. Upon application of voltage, negatively charged analytes in the sample zone migrate toward the anode. Upon entering the lower pH buffer, a pH-induced change in their charge state causes a reversal in the direction of electrophoretic mobility and a reduction in the EOF, resulting in a focusing at the interface between the high pH and low pH zones. Isoelectric focusing is an example of this class.

Isotachophoresis may also be used to focus samples within a separation capillary. Single column ITP-CE (isotachophoresis-capillary electrophoresis) involves the use of a discontinuous buffer system to create isotachophoretic separation conditions prior to the application of an electric potential. The sample is sandwiched between a leading electrolyte and a trailing or terminating electrolyte. Upon application of the potential, the analytes become stacked into discrete zones and progress down the separation capillary. Analyte concentration is uniform within a given zone, and hence analytes are detected as broad plateaus or steps. In transient isotachophoresis, the volumes of leading electrolyte and trailing electrolyte used are small, and the discontinuous buffer system disappears because the trailing electrolyte overtakes the leading electrolyte. The buffer thereby becomes continuous, and the stacked zones are separated electrophoretically as they pass through the remainder of the separation capillary, with a small but advantageous amount of dispersion that results in the production of well-resolved peaks, instead of plateaus as in nontransient ITP.

The ITP-CE can be a simple three buffer system, where leading electrolyte is first introduced into the separation capillary, followed by the sample, followed by the trailing electrolyte. The trailing electrolyte is selected such that its mobility is preferably higher at one pH and lower at another pH than the mobility of all charged analytes of interest in the sample. Or the ITP-CE can be a "two buffer" system, wherein a background electrolyte is first introduced into the separation capillary, followed by a plug of leading

electrolyte, then the sample of interest, and finally more background electrolyte, which serves as the trailing electrolyte. Alternatively, the background electrolyte can serve as the leading electrolyte, in which case first the background/leading electrolyte is introduced, followed by the sample, then the trailing electrolyte, and finally the background electrolyte. A "one buffer" ITP-CE system is useful where extreme pH is needed to effect a separation. The injected sample is sandwiched between an acid (H^+) zone and a base (OH^-) zone. When the voltage is applied, the acid and base zones will migrate toward each other, forming a region of low conductivity in the middle. This will lead to moving boundary stacking conditions, which concentrate the analytes. Either the H^+ or the OH^- ions can act as the trailing electrolyte.

Pre-concentration techniques using on-line pre-column concentrators filled with adsorptive material have also been developed. Concentration by adsorption has the additional benefit of facilitating "clean up" of samples contaminated with excessive amounts of salts, detergents, and denaturants. These agents are known to affect the reproducibility of separation by capillary electrophoresis and can interfere with analyte detection, rendering the technique useless for even qualitative analysis of the various molecular species within a sample.

As used herein in connection with the description of capillary electrophoresis applications, the term "on-line" means that the device is disposed within or the technique is conducted within the CE system, in electrical contact with the separation capillary, such that the device or technique is subjected to any electrical potential applied to the separation capillary. Thus, where an on-line precolumn concentrator or other type of sample preprocessor is used, the electric potential is not just placed across the two ends of the separation capillary, but rather is applied to the combination of the sample preprocessor and the separation capillary, such that it spans both. More generally, and in connection with other types of applications described herein, a reference to a sample preprocessor connected "on-line" means that the sample preprocessor is in physically and/or electrically continuous with the device or system used to process, separate, analyze or detect a preprocessed analyte.

Concentration (sometimes referred to herein as preconcentration) and/or sample preprocessing can be achieved with the various precolumns known in the art, but the effectiveness of the electrophoretic separation is compromised. Considerable peak broadening is commonly observed (See, e.g., A.J.J. Debets et al., J. Chromatog., 608, 151-158 (1992)). Moreover, the coatings and solid phase packing materials employed strongly affect the electroosmotic flow in the separation capillary, producing wide variability in analyte migration times. This particularly troublesome problem is associated with the reversal of EOF produced by preconcentrators known in the art and virtually precludes routine analysis of dilute clinical samples. The strength of this reverse electroosmotic flow appears to vary with the type of packing material used, the nature of the analyte, the bed volume of the packing material, the amount of analyte adsorbed, and the choice of elution solvent (A.J. Tomlinson et al., J. High Resolution Chromatog., 17, 1-5 (1994)), and is not readily amenable to quantification or standardization. Nontransient ITP has been used in conjunction with an on-line pre-column concentrator fabricated from an inert capillary tube, in an effort to focus analytes that have been eluted via electrodesorption; however, the problems produced by the reversal of EOF associated with on-line preconcentrators remain (V. Kasicka et al., J. Chromatog., 273, 117-128 (1983)).

The sample preprocessor provided by the invention addresses many of the problems associated with conventional on-line solid phase adsorbing "pre-columns". Minimizing the bed volume of the adsorptive phase by using a membrane allows reduction in the volume of eluent required for the effective removal of analytes from the adsorptive phase. A higher recovery of analytes in micro- or nanoscale applications is achieved. In capillary electrophoresis, this feature, coupled with the increased hydrodynamic flow through the membrane and the reduced ion impedance upon electrophoresis, results in a more reproducible EOF. Moreover, even though the use of a membrane instead of solid phase particulate matter reduces the bed volume (as measured by the accessible adsorptive surface area), the higher adsorptive capacity of a membrane phase affords loading of large volumes (>100 µl) of dilute sample solutions.

The sample preprocessor of the invention comprises a container having a first port, a second port, and an interior space

partitioned by at least one liquid permeable membrane into a first compartment comprising the first port and a second compartment comprising the second port, so that a liquid that enters the interior space through the first port and exits the interior space through the second port traverses the membrane. The membrane preferably comprises a sample processing material.

Samples appropriate for analysis include any liquid sample containing one or more molecular species of interest, referred to herein as a sample component or analyte. Samples are typically introduced into the first port and brought into contact with the sample processing material, where they are preprocessed as described in more detail below. Preprocessed analytes and/or other sample components are typically removed or eluted through the second port. In certain applications, it may be desirable to elute the analytes through the first port.

During an electrophoretic separation, an electrically conductive buffer is introduced into the container. This buffer comes into physical and, hence, electrical contact with the sample processing membrane. The use of a membrane reduces or eliminates problems associated with EOF reversal. Furthermore, when used in conjunction with an analyte focusing technique, as described below in a preferred method for using the preseparation processor, the problem of peak broadening associated with the elution of adsorbed analytes is largely circumvented.

The container can be fabricated from a material to which analytes do not adhere. It can be made of a metal, metalloid, glass, ceramic, graphite, organic polymer, or a composite of the foregoing materials such as a graphite-spiked polymer. Preferably, a fluorinated hydrocarbon polymer such as polyfluorotetraethylene (Teflon™) is used to fabricate the container.

The utility or effectiveness of the system of the present invention is not dependent on any particular shape of container. The container may, for example, be shaped as a cylinder, a sphere, or a box as long as it has a first port and a second port. Any configuration capable of containing the material used to concentrate or otherwise preprocess the sample is acceptable. Preferably, the container is tubular and each of the first and second compartments is cylindrical. The length of the tube is typically between about 1 mm and 10 mm. A

wide range of tube bore diameters is useful in the present invention, depending upon the type of material used to preprocess the sample and the type of sample to be analyzed. A microbore tube is preferred, such that the bore diameter is less than about 1000 μm in diameter, more preferably less than about 500 μm in diameter. Particularly convenient is 22 ga stainless steel (Type 304 L) tubing. Commercially available microbore capillary electrophoresis tubing can also be used; the bore diameters of microbore capillary electrophoresis tubing in common use are about 20-200 μm.

When it contains sample processing material, the container is also referred to herein as a microcolumn. The sample processing material can be used to concentrate, wash, or otherwise preprocess a sample prior to, or during, electrophoretic separation or other analytical application. In a preferred embodiment of the invention, the sample processing material is an adsorptive material that has an affinity for an analyte of interest present in a sample to be analyzed, and is thereby useful for concentrating said analyte and/or for removing unwanted materials from the sample such as detergents and salts. Other types of sample preprocessing that can be carried out with the sample preprocessor of the invention include analyte cleavage, fluorescence or radioactive labeling or other chemical derivatization, oxidation or reduction, catalysis of a chemical reaction, or the like, and can be enzymatic or nonenzymatic.

Membranes are particularly preferred because of ease of fabrication of the microcolumn, and the minimization of dead volume, which increases resolution. Also, in capillary electrophoresis applications, membranes appear to reduce the problems associated with the reversal of EOF. A membrane is typically in the form of a thin, porous, mechanically stable shaped film, i.e., a disk. The membrane can itself be made of a material that can process the sample or it can incorporate a variety of materials, such as known chromatographic support materials, that effect the processing. In a particularly advantageous form, the membrane is composed of a chemically inert organic polymer matrix, such as polytetrafluoroethylene (PTFE), embedded with adsorbent particles, such as silica. A typical membrane used to practice the invention has a thickness of less than about 1 mm, whereas particulate packing

material typically occupies a length of about 0.1 to 10 mm, preferably a length of about 0.1 to 1.0 mm.

In a preferred embodiment of the invention, the sample processing material is a membrane having a polytetrafluoroethylene (i.e., PTFE or Teflon™) lattice structure. In a particularly preferred embodiment, the membrane is impregnated with solid phase beads. An EMPORE™ membrane (3M Company, St. Paul, MN) is an example of a Teflon™ lattice membrane impregnated with particulate solid phase beads comprising C_{18} alkyl groups. EMPORE™ is available in large sheets about 500 μm thick. The sheets can be rolled out or flattened into thicknesses of less than about 100 μm, preferably less than about 50 μm. Small discs suitable for insertion into the sample preprocessor of the invention can be punched out of the sheets with a hypodermic needle.

The use of a liquid-permeable membrane as the sample processing material is particularly preferred for use in capillary electrophoresis or other techniques, such as various forms of chromatography, where hydrostatic pressure is used to move the sample through the sample preprocessor. Separation efficacy is a function of hydrodynamic flow rate, and particulate matter may cause increased back pressures that limit the flow rate that can be used. The use of a membrane in the sample preprocessor thus allows operation of the system over a larger range of pressures.

The sample processing material preferably includes silica or a reactive organic polymer. In a preferred embodiment of the invention useful for concentrating or decontaminating a sample that contains at least one analyte, the sample processing material is an adsorptive material with an affinity for an analyte. The adsorptive material can be derivatized with functional groups that adsorb, adhere, or otherwise bind, covalently or noncovalently, an analyte of interest. The binding may be specific, as in an antibody/antigen interaction, or nonspecific, as in a hydrophobic interaction. Derivatization with an aliphatic C_{18}, C_8, or C_4 hydrocarbyl (i.e., alkyl) group is particularly advantageous.

In another preferred embodiment of the invention useful for chemically preprocessing the sample prior to or during electrophoretic separation, the sample processing membrane is derivatized with a molecular species selected to carry out the desired

preprocessing of the analyte. For instance, a proteolytic enzyme such as trypsin, or a disulfide isomerase, such as thioredoxin or protein disulfide isomerase, may be immobilized on a membrane. A molecular weight filter may be used to filter out molecules having a molecular weight under the filter threshold.

Examples of derivatizing agents useful for concentrating or chemically preprocessing analytes include functional groups, ligands, or other molecular species such as a C_{18}, C_8, C_4 hydrocarboyl or other aliphatic or hydrophobic group; an anionic or cationic group; an antibody or antigen; an amino acid; a peptide; a coenzyme or other biological cofactor, such as biotin; a lectin, glycoprotein, polysaccharide or other carbohydrate-containing moiety; a nucleotide or nucleic acid; a protein or enzyme, such as Protein A, or Protein G; a metal or metal chelate; a molecular superstructure such as C_{60} buckminsterfullerene; or a dye, for example, blue dextran or Cibacron Blue 3GA (Sigma Chemical Company, St. Louis, MO). It is to be understood that the preceding list in no way limits the choice of molecules that can be used to derivatize the sample processing material of the membrane. Likewise, the sample processing material can be underivatized.

Two or more membranes can be placed in series inside the tube, allowing for multiple-step purification, processing and/or separation strategies with minimal dead volume and sample handling. Thus, the invention provides a sample preprocessor wherein a plurality of membranes partitions the interior space into a plurality of compartments so that a liquid that enters the interior space through the first port and exits the interior space through the second port traverses each of the membranes. Alternatively, two or more membranes can be abutted, so that the container is divided into two compartments.

In a preferred embodiment, at least one membrane is a chemically inert organic polymer matrix embedded with adsorptive particles. In another preferred embodiment, at least one membrane is a molecular weight filter. For example, a hydrophobic membrane or resin can be used in conjunction with an anion exchange membrane or an immunoadsorbant membrane, or a C_{18} derivatized membrane can be used in conjunction with a cationic derivatized membrane, a membrane derivatized with a monoclonal antibody or a molecular

weight filter that allows passage of only those molecules having a molecular weight about equal to or less than a defined molecular weight cutoff.

It may be helpful or necessary to confine, retain or immobilize the membrane in the microcolumn. Accordingly, the sample preprocessor of the present invention further includes confinement means for confining the membrane in the container. Means for confining the membrane is used so as to not impede the flow of liquids through the container. Preferably, one or more frits, plugs, gas or liquid bubbles, physical crimps, constrictions, laser treatments, supports or heat compressions of the container, or any combination thereof, is used to position the membrane(s) within the container.

More preferably, a frit or plug is used. Any material sufficiently porous to allow liquid to pass while containing the sample processing material can be used to construct a frit or plug. Silica, polymer, ceramic or metal are preferred fabrication materials. Examples of preferred materials include wool, polyethylene, zirconia, refractory metals, glass, or composite thereof. Silica or polymer wool is particularly preferred. A useful polymer is one that has been fabricated in porous form during curing or processing.

Small particles derived from wools or other such materials can be used to form the frit or plug. A frit is typically less than about 1 mm thick. Alternatively, the sample processing material is immobilized by narrowing, constricting or crimping the internal wall or surface of the tube to confine the sample processing material, as by applying a mechanical force or laser treatment to the container wall, or by subjecting it to heat treatment.

Also provided by the invention is a sample preprocessing assembly which adds several useful features to the sample preprocessor of the invention. These features include sample introduction means in fluid communication with the first port of the container to form an inlet connection, sample removal means in fluid communication with the second port of the container to form an outlet connection, and a sealant for sealing the inlet and outlet connections so formed to prevent the leakage of liquid. The sample removal means permits removal of a preprocessed analyte or other component from the sample preprocessor. When used in capillary

electrophoresis, the sample preprocessing assembly is sometimes referred to herein as a preseparation processor assembly.

The sample preprocessor used in the sample preprocessor assembly is as disclosed above. In a particularly preferred embodiment, the sample preprocessor assembly contains a plurality of membranes that partition the interior space into a plurality of compartments so that a liquid that enters the interior space through the first port and exits the interior space through the second port traverses each of the membranes.

An example of a convenient means for introducing and removing samples as provided in the sample preprocessor assembly is microbore capillary electrophoresis tubing. Typically, a commercially available microbore capillary electrophoresis tube, which typically has an internal diameter of about 20-200 μm, is used. Preferably, the tubing is fabricated from a material such as silica, glass, ceramic, or a chemically inert organic polymer such as polytetrafluoroethylene (PTFE), polyetheretherketone (PEEK), polyimide, or polyethylene.

When the container is in the shape of a cylindrical tube with approximately the same diameter as the microbore capillary electrophoresis tubing, the microbore tubing can be connected to the microcolumn by directly abutting the microbore tubing to the cylindrical tube or inserting it into the ports of the cylindrical tube. A direct route for the flow of fluids from the microbore capillary electrophoresis tubing into the microcolumn is thus provided, minimizing the dead volume in which the sample might pool. Sample pooling compromises recovery and resolution, and is preferably avoided. The dead volume to be minimized includes the volume accessible to the liquid outside of the tube and the sample introduction means, such as the volume around the outer surface of the sample introduction means but nonetheless inside the sealed inlet connection. Microbore capillary electrophoresis tubing used as means for introducing and removing samples can be externally coated with a thin layer of organic polymer such as curable epoxy or polyimide for strength. Typically this coating is less than about 50 μm thick.

Preferably, the sealant used to seal the connections is a chemically resistant organic polymer, such as epoxy, polyimide,

polyvinyl chloride (PVC), polyethylene, or polypropylene, or glass, ceramic, or a composite of the foregoing materials. More preferably, a chemically resistant organic polymer is used; most preferably, the chemically resistant organic polymer is a curable epoxy resin.

Chemically resistant materials, as the term is used herein, are those that withstand the chemicals commonly used in the art of capillary electrophoresis, such as acetonitrile, methanol, ammonium acetate, dilute acetic acid and other acids; thus any material that shows no detectable degradation during the duration of a capillary electrophoresis experiment, typically about 15 to 40 minutes, is suitable. A material that shows degradation during a typical capillary electrophoresis run and is thus unsuitable for use as a means for sealing the connections in the present invention is uncured polyimide. Some materials, e.g, some commonly available "5 minute" epoxies such as EPOXI-PATCH (Hysol Aerospace, Seabrook NH), may be suitable for single-use applications, but begin to degrade after about 1 to 3 separation experiments.

Examples of preferred materials include those that meet USP Plastic Class VI requirements (1995 United States Pharmacopeia, USP 23, National Formulary, NF 18, pp. 1699-1703 United States Pharmacopoeia Convention, Inc., 12601 Twinbrook Parkway, Rockville, MD, 20852) such as the bisphenol-novolac epoxy blend, EP42HT (MASTER-BOND, Hackensack, NJ). The previous examples notwithstanding, it is to be understood that the sealing means is not limited to any particular epoxy or organic polymer, but is to be broadly understood to encompass any means or material that provides an effective liquid seal at the inlet and outlet connections of the sample preprocessor assembly, as disclosed above.

In a particularly preferred embodiment of the invention, the sample preprocessor is constructed by inserting a circular membrane into a narrow bore (internal diameter of between about 140 to 600 μm) housing, e.g., a Teflon™ housing, wherein the diameter of the membrane is essentially equal to the internal diameter of the cylindrical housing, then inserting short (about 1-2 cm) lengths of fused silica capillary tubing into each end of the narrow bore housing to physically confine the membrane perpendicular to the long axis of the housing, serve as means for sample introduction and removal, and permit liquid flow through

the membrane. In this embodiment, sample introduction means and removal means also serve as confinement means for the membrane. Preferably, the housing is sealed onto the capillary tubing using a solvent resistant epoxy resin to prevent leakage and to provide mechanical strength.

The sample preprocessing assembly provided by the invention can further include means for attaching the sample removal means to a separation capillary. This means for attachment can conveniently take the form of one piece of organic polymer tubing of a length sufficient to allow for the effective sealing of the liquid joint between the sample preprocessor assembly and a separation capillary. Preferably, the tubing is inert, i.e., it is chemically nonreactive and is not degraded or otherwise affected by the buffers, reagents or analytes used in capillary electrophoresis. Typically, the connector is about 6-7 mm in length.

Preferably, the means for attachment is made up of an outer piece of elastic organic polymer (i.e., plastic) tubing, which provides cylindrical pressure directly over the liquid-liquid junction between the output end of the sample preprocessor and the input to the capillary electrophoresis tube, surrounding an inner piece of organic polymer tubing sufficient to supply an adequate liquid seal.

Preferably, the outer tubing is fabricated from silicone rubber, such as Dow-Corning SILASTIC, and the inner tubing is fabricated from an inert organic polymer such as polytetrafluoroethylene (PTFE), polyetheretherketone (PEEK), or polyethylene.

The present invention further provides a capillary electrophoresis system fitted with a preseparation processor or preseparation processor assembly as disclosed hereinabove, termed herein a "preprocessing capillary electrophoresis system." At least one sample preprocessor assembly is connected to the inlet end of a separation capillary, so that the sample removal means of the sample preprocessor assembly is in fluid communication with the inlet end of the separation capillary. In a preferred embodiment, the preprocessing electrophoresis system also includes electrical means for electrokinetically separating analytes of the sample preprocessor when in use. Typically, the electrical means comprises two electrodes and a power supply. The sample processing membrane of the

preseparation processor is as disclosed hereinabove and preferably includes an adsorptive material for concentrating a sample containing at least one analyte.

One or more preseparation processors or preseparation processor assemblies may be placed in series within a commercially available capillary electrophoresis system, such as the Beckman Instruments P/ACE 2100 capillary electrophoresis system (Fullerton, CA), upstream from the separation capillary, such that the second port of the container of the preseparation processor is connected to the inlet end of the separation capillary. Typically, the separation capillary is fabricated from silica and has an internal diameter of about 20-200 μm, and a length of about 10-150 cm. Preferably, the microcolumn is adjacent to the separation capillary, and the second port of the container of the preseparation processor is within about 15 cm of the inlet end of the separation capillary, more preferably within about 2 cm of said inlet end.

Where two or more sample preprocessors are used in the formation of the preprocessing capillary electrophoresis system they are connected in line adjacent to each other such that a sample that enters the first port of the first sample preprocessor and exits the second port of the last sample preprocessor traverses each of the sample processing membranes prior to entering the separation capillary. Preferably, each sample processor contains a different sample processing membrane.

To connect the sample preprocessor or sample preprocessor assembly to the separation capillary, microbore capillary electrophoresis tubing that has been coated or treated on the inside surface using various reagents such as silanizing reagents or hexadimethrine bromide (also known as polybrene) to reduce or eliminate sample adsorption on the capillary walls is preferably used.

Also provided by the present invention is a method for performing sample preprocessing and subsequent capillary electrophoresis, which method is termed herein "preprocessing capillary electrophoresis." The preseparation processor or preseparation processor assembly of the invention is connected to the inlet end of a separation capillary so as to form a preprocessing capillary electrophoresis system. The preprocessing capillary electrophoresis system preferably includes the preseparation processor

or preseparation processor assembly of the invention as disclosed hereinabove, separation capillary, means for detecting analytes during or after separation, means for applying an electric field across the system, and any wiring, valving, or tubing needed to connect the various components to one another and perform capillary electrophoresis. The preseparation processor can be pretreated, flushed, washed or cleaned with an electrically conductive buffer or any other necessary or suitable liquid to prepare it for a separation experiment.

A liquid sample containing at least one analyte of interest is introduced into the capillary electrophoresis system and brought into contact with the sample processing membrane in the container of the preseparation processor. In a preferred embodiment, the sample processing membrane comprises an adsorptive material, so that one or more analytes adheres to the adsorptive material and thereby becomes concentrated. The sample is introduced into the first port of the container. Hydrostatic or hydrodynamic methods, which work by changing the pressure of the sample relative to the pressure of the liquid in the capillary electrophoresis system, can be used to introduce the sample into the first port of the container. Hydrostatic methods of sample introduction include the use of vacuum (suction) or pressure (typically about 0.5-30 p.s.i.). Alternatively, the sample can be electrokinetically introduced into the container by applying an electrical field to induce the movement of charged sample components into the first port of the container. After contact with the sample processing material, the preprocessed sample leaves the container through the second port.

In cases where the sample processing material contains an adsorptive material with affinity for one or more analytes, analytes are removed from the container by supplying elution media capable of releasing an analyte of interest from the adsorptive material. For example, an organic solvent can be used to elute an analyte from a C_{18}-derivatized adsorptive material, or a buffer containing a cleavage agent can be used to elute an analyte that has become covalently attached to an adsorptive material. Elution can be effected using hydrostatic methods such as suction (vacuum), pressure (typically about 0.5-30 p.s.i.) or gravity injection. Alternatively, analytes can be eluted using an electrokinetic method such as electrodesorption (V.

Kasieka et al., J. Chromatog., 273, 117 (1983), incorporated herein by reference).

Preprocessed analytes can be separated using protocols commonly used in the art of capillary electrophoresis. At least one electrically conductive buffer is introduced into the capillary electrophoresis system, and an electric field of typically about 1-60 kV is applied across the preprocessing capillary electrophoresis system. The separated analytes are detected according to methods well-known in the art of capillary electrophoresis. Examples of convenient detection methods include monitoring optical absorption or optical fluorescence, mass spectrometry, electrochemical techniques, conductimetric techniques, monitoring radioactivity, and following changes in refractive index.

Multiple sample elution buffers can be used in the system and the method of the present invention. In a multiple-step purification, processing or separation experiment, several different buffers, differing in composition, pH, salt or organic content, can be used. Moreover, the elution, separation, and detection steps can be repeated as needed for complete analysis of the analytes contained in a given sample.

In a preferred method for sample preprocessing and subsequent capillary electrophoresis, an analyte focusing technique is used in conjunction with the preseparation processor of the invention prior to detection of processed analytes. Preferably, the analyte focusing technique is pH dependent sample focusing, isotachophoresis (ITP), or field amplification.

In a particularly preferred embodiment, the sample processing material comprises an adsorptive material for concentrating a sample, and transient ITP is used to focus the concentrated analytes. The pre-processing capillary electrophoresis system is first filled with an electrically conductive buffer, after which a liquid sample containing at least one analyte is introduced into the first port of the container so as to contact the adsorptive material therein. Elution of adsorbed analytes from the container is preceded by injection of an aqueous alkaline or base (OH^-) plug into the first port of the container and followed by injection of an aqueous acid (H^+) plug into the container. The volumes of the acid and alkaline plugs must be small enough to avoid overheating due to the

increased electric field in the plugs. Preferably, the volumes are less than about 1 µl, more preferably, less than about 500 nl, most preferably, less than 200 nl.

The acids and bases employed in transient ITP are preferably volatile, such that they are compatible with analyte detection using mass spectrometry. More preferably, the base is about 0.01% to 0.5% aqueous ammonium hydroxide solution, most preferably about 0.1% aqueous ammonium hydroxide solution. The acid is preferably about 0.1% to 5% aqueous acetic acid, most preferably about 1% aqueous acetic acid. Alternatively, the acid employed in ITP can be a combination of aqueous ammonium acetate and aqueous acetic acid, such as NH_4OAc: 1% AcOH. The eluted analytes are then electrophoretically separated and detected as described above.

It is to be understood that the while the sample preprocessor of the invention is especially suited for use as a preseparation processor in capillary electrophoresis, it is equally useful in a much broader range of analytical and preparative chemical techniques. Thus, the invention provides a method for using the sample preprocessor either off-line, or on-line with any suitable system or device for processing, separating, detecting or analyzing analytes. As described above in connection with its use as a preseparation processor in capillary electrophoresis, the sample preprocessor is useful for all types of sample decontamination, detoxification, desalting, concentration and/or preprocessing. The type of preprocessing effected by the sample preprocessor is determined by the nature of the sample processing material contained therein, as described in detail above.

Off-line use of the sample preprocessor may be accomplished by hydrostatically injecting the sample into the sample preprocessor and moving it hydrodynamically through the sample processing material, preferably a membrane, such that the analyte of interest is adsorbed onto the sample processing material. The sample may also be loaded into the sample preprocessor electrokinetically.

The preloaded sample preprocessor can then be connected in-line to a capillary electrophoresis system or other system or device for analyte processing, separation, detection or analysis, and the immobilized analyte can be either processed further while immobilized or eluted using an effective elution agent, such as an

aqueous buffer or salt solution, an organic solvent, or a mixture thereof.

When the sample preprocessor contains two or more membranes, the method of the invention may at times be advantageously practiced by loading the sample off-line through the first port followed by a reversal of the flow direction prior to analyte elution. For example, when the liquid sample first contacts a molecular weight filter, sample components such as large proteins and DNA molecules having a molecular weight higher than the molecular weight cutoff value of the filter may build up on the filter surface. Analytes of interest passes through the molecular weight filter and can then adsorb onto a second filter comprising an adsorptive material that concentrates the analyte. Prior to elution of the immobilized concentrated analyte from the second membrane, liquid flow may be reversed through the sample preprocessor in order to remove the buildup of high molecular weight species.

In a particularly advantageous process, several sample preprocessors are prepared in advance or are used simultaneously in rotation; i.e., while one or more sample preprocessors are in use off-line for adsorbing, preconcentrating or preprocessing analytes, one or more other sample preprocessors containing the preprocessed analytes are concurrently subjected to on-line processing, separation, detection or analysis. This rotation allows the on-line processing, separation, detection or analysis device or system to be run continuously without any downtime for sample adsorption, preconcentration or preprocessing.

A sample that has been preprocessed off-line using the sample preprocessor of the invention may then be analyzed using any technique of interest, such as capillary electrophoresis, high performance liquid chromatography (HPLC), micro-bore or capillary HPLC, capillary electrochromatography (CEC), microdialysis, nuclear magnetic resonance (NMR), and various mass spectrometric techniques such as electrospray ionization-mass spectrometry (ESI-MS), microspray or nanospray ESI-MS, matrix-assisted laser desorption time of flight mass spectrometry (MALDI-TOF-MS), magnetic sector mass spectrometry, quadrupole mass spectrometry, quadrupole iontrap mass spectrometry, fourier transform ion cyclotron resonance mass spectrometry, and the like. Thus, the

invention further provides a method for analyzing sample having been preprocessed off-line using the sample preprocessor of the invention.

The method provided by the invention for on-line use of the sample preprocessor of the invention applies to its use on-line with any suitable system or device, including, for example, in addition to a capillary electrophoresis system as described above, a high performance liquid chromatography (HPLC) system, a micro-bore or capillary HPLC, a capillary electrochromatography (CEC) system, mass spectrometers of all types, such as electrospray ionization-mass spectrometers (ESI-MS), microspray or nanospray ESI-MS, and matrix-assisted laser desorption time of flight mass spectrometers (MALDI-TOF-MS), and microdialysis.

For example, electrospray ionization-mass spectrometry (ESI-MS) is particularly intolerant of salt present in samples to be analyzed. Therefore, such contaminants must be removed prior to analysis high-salt samples, such as those that are biologically-derived, eluted prior to analysis using ion-exchange chromatography, or obtained from solution using a salting in or out process. Conventionally, "desalting" would be achieved using a macro-scale device, such as a solid phase extraction cartridge, preparative or semi-preparative HPLC, and the like. In these conventional methods, analyte losses to exposed surfaces such as the beads of the solid phase, the cartridge or container walls, and the like, are often substantial. Using the sample preprocessor or the invention, either off-line or on-line, minimizes the exposed surfaces with which the sample comes into contact due to its microscale. The use of a membrane instead of a solid phase packing material further reduces the amount of exposed surface.

The sample preprocessor of the invention is particularly suited for use either off-line or on-line to preprocess samples prior to analysis using the newly developed technique of micro-spray or nano-spray ESI-MS. Microspray or nanospray ESI-MS use an extremely slow sample flow rate (20-30 nL/minute). Because sample flow rates are considerably slower than those of the conventional ESI source, sample consumption is less. The slow flow rate produces very small initial droplets at the tip of the ESI needle, which enhances analyte ionization efficiencies by permitting a more efficient

desolvation process. The geometry of the micro/nanospray ESI source allows the spray needle to be moved closer to the inlet of the mass analyzer than in conventional ESI. Coupled with the small droplet size, this ensures a higher transmission of analyte ions into and through the mass spectrometer. M.S. Wilm et al., Int. J. Mass Spectrom. Ion Proc., 136, 167-180 (1994); M. Mann et al., Anal. Chem., 66, 4390-4399 (1994).

In a preferred method, the sample preprocessor is inserted close to the spray needle of the ESI source. Sample losses are prevented and a more efficient delivery of analytes to the mass spectrometer is effected thereby.

Matrix-assisted laser desorption time of flight mass spectrometry (MALDI-TOF-MS) is not as sensitive to salt concentration as is ESI-MS, but analysis of samples containing extremely high salt concentrations (for example, biologically-derived analyte mixtures) remains problematic. A high level of salt interferes with sample/matrix co-crystallization processes and precludes successful analysis of such samples. Sample cleanup, desalting and/or preprocessing using the sample preprocessor of the invention, preferably having as its sample processing material a membrane phase, would be advantageous since sample losses to the sample preprocessor are minimized compared with the use of standard macro-scale, more conventional devices, such as solid phase extraction cartridges.

The sample preprocessor of the invention is also ideally suited for use either on-line or off-line in sample preconcentration and/or clean-up prior to capillary electrochromatography (CEC). CEC is essentially a hybrid between HPLC and capillary electrophoresis. It is performed in a capillary that is filled with a typical HPLC stationary phase. Analyte migration is then driven electrophoretically. CEC can afford different separation selectivities (particularly for ionic species) than attainable with conventional HPLC. However, the small internal diameter of the capillaries limits the amount of sample that can be analyzed. Hence, CEC suffers the same poor concentration limits of detection as capillary electrophoresis. I. Grant, Capillary Electrophoresis Guidebook--Principles, Operation and Applications (K.D. Altria, ed.), in Methods in Molecular Biology, Vol. 52 (Hanna Press Inc., NJ) 197-209 (1996).

In a preferred method, the sample preprocessor of the invention having as its sample processing material a membrane is used on-line to concentrate a sample prior to analyte separation. Preconcentration using the sample preprocessor of the invention is expected to overcome the poor concentration sensitivity attendant to analytical separation techniques such as capillary electrophoresis and CEC.

Other uses of the sample preprocessor of the invention include use as a micro-guard column for small scale (e.g., micro-bore or capillary) HPLC, and to concentrate and prepare samples extracted from tissues by micro-dialysis prior to analysis by, for example, any of the aforementioned techniques.

The apparatus and method of the invention can be used for both analytical (small-scale) and preparative (larger-scale) applications. Small scale applications include, for example, the preprocessing of very dilute samples where preconcentration of the analyte (by way of adsorption to the sample processing material) is important for successful detection. As an example of a larger scale application, samples containing about 40 pmol of protein can be preprocessed without sample breakthrough using the sample preprocessor disclosed in Figure 2 having an EMPORE™ membrane as the sample processing material. That is, aabout 40 pmol or more of protein will adsorb onto the membrane before saturating the membrane.

A preferred embodiment of the sample preprocessor assembly of the present invention is shown in Figure 1. A tubular container (1) having an inner surface (2) and an outer surface (3) is constructed from a cylindrical tube of polytetrafluoroethylene (PTFE), for example. The container (1) has a first port (4) and a second port (5) and surrounds a sample processing material (8) and two retaining frits (9), which confine the motion of the sample processing material (8). In this embodiment, the sample processing material (8) constitutes adsorptive particles commonly used in high performance liquid chromatography (HPLC). The sample processing material (8) is disposed inside the tube (1) such that the processing material is in contact with the inner surface (2) of the container (1) when in use during an electrophoretic separation. The frits (9) are fabricated from a polyethylene wool, for example. A piece of microbore glass tubing

(10) of the type that is commonly used in capillary electrophoresis is inserted into the second port (5) of the tube (1) so as to provide means for removing a preprocessed sample from the second port (5) of the tube (1). An outlet connection (6) is thereby formed at the juncture of the microbore tubing (10) and the second port (5). Likewise, a piece of microbore glass tubing (11) of the type that is commonly used in capillary electrophoresis is inserted into the first port (4) of the tube (1) so as to provide means for introducing a sample into the first port (4) of the tube (1). An inlet connection (7) is thereby formed at the juncture of the microbore tubing (11) and the first port (4). The microbore tubing (10, 11) is constructed from silica, for example, and is coated with a thin external organic polymer layer (12) such as polyimide, for example, for physical strength.

The microbore tubing (10, 11) is placed within the tube (1) in a manner adjacent to and directly abutting the frits (9), which allows the ready flow of liquid through the frits (9) and sample processing material (8). The assembly comprising (1), (3), (2), (8), (9), portions of (10) and (11), (4), (5), (6), and (7), is encased in a chemically resistant, curable epoxy coating (13), which provides physical strength and also serves to seal the liquid joints at the outlet connection (6) and the inlet connection (7). A small connector consisting of a length of organic polymer tubing (14) sufficient to allow for the effective sealing of the liquid joint between the sample preprocessor assembly and a length of capillary tubing (15) is attached to the opposite end of the microbore tubing (10). This connector (14) facilitates the connection of the sample preprocessor to the desired separation capillary or other system or device.

Figure 2 shows another preferred embodiment of the sample preprocessor assembly of the present invention. This embodiment is identical to that shown in Figure 1, with two exceptions: first, the sample processing material consists of a membrane (16), and second, frits are unnecessary for confining and positioning the membrane material, although they may be included if desired. The two pieces of microbore glass tubing (10, 11) are inserted into the second port (5) and first port (4), respectively, of the tube (1) adjacent to and directly abutting the adsorptive membrane (16), so as to direct the flow of liquid comprising the analyte through the adsorptive membrane (16).

Another preferred embodiment of the sample preprocessor assembly is shown is Figure 3. In this embodiment, the tube (1) contains two membranes (17a, 17b). The membranes (17a, 17b) are derivatized differently so as to facilitate two step purification strategies. As in the embodiment shown in Figure 2, frits are unnecessary for confining the membranes, but may be included if desired. The two pieces of microbore glass tubing (10, 11) are inserted into the second port (5) and first port (4), respectively, of the tube (1) adjacent to and directly abutting the membranes (17a, 17b) so as to allow direct the flow of liquid comprising the analyte through the stacked membranes (17a, 17b).

Figure 4 shows another preferred embodiment of the sample preprocessor assembly of the invention. This embodiment is identical to that shown in Figure 1 except that in this embodiment the connector (designated (14) in Figure 1) is made from an outer piece of organic polymer tubing (24) with a high elasticity, and an inner piece of organic polymer tubing (23) effective to supply an adequate liquid seal. The outer elastic polymeric based connection tube (24) provides cylindrical pressure directly over the liquid-liquid junction between the output end of the sample preprocessor (10) and the input to the capillary electrophoresis tube (15). The inner connection tube (23) consists of a length of inert organic polymer tubing sufficient to effectively seal the liquid joint between the sample preprocessor and the capillary electrophoresis tubing.

A preferred embodiment of the sample preprocessor of the invention is shown in Figure 11. A tubular container (1) as in Figure 1 has an interior space (25) partitioned into two compartments (26a, 26b) by a sample processing membrane (16). A liquid sample enters the first compartment (26a) of the container (1) through a first port (4), traverses the membrane (16), then exits the second compartment (26b) of the container (1) through a second port (5).

Figure 12 shows a preferred embodiment of a sample preprocessing system of the invention wherein liquid flow through the preprocessor is accomplished by use of a pressurized bomb. The sample preprocessor is shown in Figure 11. It includes a tubular container (1) having an interior space (25) partitioned by a sample processing membrane (16) into a first compartment (26a) comprising the first port (4) and a second compartment (26b) comprising the

second port (5). The sample processing membrane (16) is preferably composed of EMPORE™ styrene divinyl benzene (SDB) copolymer membrane. Two pieces of microbore glass tubing (10,11) are inserted into the second port (5) and first port (4), respectively, of the tube (1) adjacent to and directly abutting the adsorptive membrane (16) so that liquid entering the interior space (25) through the first port (4) and exiting through the second port (5) traverses the adsorptive membrane (16). The inlet connection (7) and outlet connection (6) are sealed in a chemically resistant, curable epoxy coating (13) which prevents leakage of liquid and also provides physical strength. The microbore tubing (11) inserted into the first port (4) is connected to a pressure source (27), such as a nitrogen pressure bomb. The microbore tubing (10) inserted into the second port (5) is connected to a small connector consisting of a length of organic polymer tubing (14), such as a polyethylene sleeve, sufficient to allow for the effective sealing of the liquid joint between the sample preprocessor assembly and a length of capillary tubing (15). This length of capillary tubing (15) may be connected to a suitable sample processing devise or analyte detector, such as a Bruker Biflex time of flight mass spectrometer (Bruker Instruments, Billerica, MA).

Figure 13 shows a preferred embodiment of the present invention utilizing two sample preprocessors in series in a preprocessing capillary electrophoresis system. Two sample preprocessors (31, 33) each containing a sample processing membrane (34, 35) are connected in line adjacent to each other such that a sample that enters the first port (30) of the first sample preprocessor (31) and exits the second port (32) of the second sample preprocessor (33) traverses each of the sample processing membranes (34, 35) prior to entering the separation capillary (36).

Objects and advantages of this invention will now be illustrated by the following examples. However, the particular materials and amounts thereof recited in these examples, as well as other conditions and details, are to be interpreted to apply broadly in the art of capillary electrophoresis and should not be construed to unduly limit this invention.

Example 1. Comparative Separations of Concentrated and Dilute Peptide Mixtures: Preseparation Processor vs. Standard CE

Preseparation concentration capillary electrophoresis (PC-CE) using a preseparation processor (Figure 1) packed with polymer-based C_{18} beads (Polymer Laboratories, Amherst, MA) as described in Example 1B, below, was used to separate a standard peptide mixture containing varying concentrations of peptide. Analyses were performed on a modified Beckman Instruments P/ACE 2100 capillary electrophoresis system (Fullerton, CA) coupled to an IBM compatible Reason Technology 486 personal computer (Rochester, MN) using system control and data capture and analysis by SYSTEM GOLD software (Beckman Instruments, Fullerton, CA). Peptide elution was monitored at 200 nm.

Acetic acid was obtained from Aldrich Chemical Company (Milwaukee, WI). HPLC grade methanol and water were obtained from Baxter/Burdick & Jackson (Muskegon, MI). Ammonium acetate and trifluoroacetic acid (TFA) were obtained from Sigma Chemical Company (St. Louis, MO). The peptide mixture used in these experiments was the peptide standard mixture obtained from Biorad (Cambridge, MA), consisting of bradykinin, angiotensin II, α-melanocyte stimulating hormone, thyrotropin releasing hormone, leuteinizing hormone releasing hormone, bombesin, oxytocin, leucine enkephalin, and methionine enkephalin. The separation runs were conducted in the order described below in Examples 1A through 1D.

Example 1A. Standard CE of Peptide Mixture: Concentrated Sample (50 µg/ml).

Capillary electrophoresis without preconcentration (standard CE) was used to separate a peptide mixture (50 µg/ml) using a 7 second pressure (0.5 p.s.i.) injection. Running conditions: bare (uncoated) silica capillary, 50 µm internal diameter (i.d.) x 67 cm length, 60 cm to detector (total separation length of 67 cm); separation voltage of 21.4 kV (4.5 µAmps) across the capillary which was maintained at 24°C; separation buffer of 2 mM ammonium acetate in 1% acetic acid (175 mM), pH 2.6. All nine proteins were detected within 15 minutes as very sharp peaks.

Example 1B. PC/CE of Peptide Mixture Using Preseparation Processor: Dilute Sample (1 µg/ml), With and Without Analyte Stacking.

Preconcentration capillary electrophoresis (PC-CE) was used to separate a dilute peptide mixture (1 µg/ml). The preseparation processor used in the following two separations consisted of a 0.5 mm column fabricated from 22 ga 304 L stainless steel tubing, 1 cm long packed with Polysorb RP, 60-100 µm beads (Polymer Laboratories, Inc., Amherst, MA) and fitted with polyethylene wool plugs.

A separation utilizing preconcentration electrophoresis was performed using the following protocol. Running conditions: bare (uncoated) silica capillary, 50 µm i.d. x 67 cm length, 60 cm to detector including preseparation processor, placed 1.5 cm from the inlet port; separation voltage of 21.4 kV (2.2 µAmps) for 60 minutes across the capillary which was maintained at 24°C; separation buffer of 2 mM ammonium acetate in 1% acetic acid (175 mM), pH 2.6.

Prior to sample loading, a 2 minute rinse was performed with separation buffer. The peptide mixture was introduced into the preseparation processor at high pressure over 30 seconds, then washed 3 minutes with the separation buffer. The sample was eluted from the preseparation processor at high pressure for 12 seconds with 80% methanol, 20% dilute aqueous acetic acid (1%), 0.1% TFA, after which it was subjected to a short (12 second) high pressure push with separation buffer. Following the electrophoretic separation, as described above, the unit was washed for 2 minutes with 100% methanol and re-equilibrated with separation buffer for 5 minutes to prepare for the next run. All proteins were detected (in seven peaks, with co-elution of two components) in about 35 minutes. Peaks showed tailing (adsorption onto capillary wall) suggesting possible peptide denaturation. Recovery was estimated at 88%.

Next, a separation utilizing preconcentration electrophoresis with the preseparation processor plus analyte stacking was performed using the protocol described in the preceding two paragraphs with two modifications. First, base (0.1% ammonium hydroxide) was injected for 6 seconds immediately prior to sample elution from the preseparation processor. Second, the high pressure

push with separation buffer following sample elution from the preseparation processor was applied for only 6 seconds, rather than 12 seconds. All nine peptide components resolved; some degradation of sample was evident by a multiplicity of peaks. All peaks were very sharp, with minimal tailing. Recovery was estimated at 88%.

Example 1C. Standard CE of Peptide Mixture--Dilute Sample (1 µg/ml). Standard CE was used to separate a peptide mixture (1µg/ml) using a 7 second pressure (0.5 p.s.i.) injection. Running conditions: bare (uncoated) silica capillary, 50 µm x 67 cm length, 60 cm to detector (total separation length of 67 cm); separation voltage of 21.4 kV (4.5 µAmps) across the capillary which was maintained at 24°C; separation buffer of 2 mM ammonium acetate in 1% acetic acid (175 mM), pH 2.6. Baseline only was produced; peptides were undetectable at this level.

Example 1D. Standard CE of Peptide Mixture--Concentrated Sample (50 µg/ml). Standard CE was used to separate a peptide mixture (50 µg/ml) using a 7 second pressure (0.5 p.s.i.) injection. Running conditions: bare (uncoated) silica capillary, 50 µm x 67 cm length, 60 cm to detector (total separation length of 67 cm); separation voltage of 21.4 kV (4.5 µAmps) across the capillary which was maintained at 24°C; separation buffer of 2 mM ammonium acetate in 1% acetic acid (175 mM), pH 2.6. Peaks were smeared, due to protein adsorption to capillary, and deterioration of sample. Recovery was about 60% compared to initial electropherogram. Upon cleaning with NaOEt (0.5 M NaOH-50% ethanol) the peaks sharpened and recoveries increased to 90+%.

Example 2. Improved Separation Resolution with the Preseparation Processor using Transient Isotachophoresis

Method. Preseparation concentration capillary electrophoresis using mass spectrometric detection of analytes (PC-CE-MS) was performed on a Beckman Instruments P/ACE 2100 capillary electrophoresis system (Fullerton, California) modified for use with electrospray mass spectrometry that was coupled to an IBM compatible Reason Technology 486 Personal Computer (Rochester,

Minnesota) using system control and data capture and analysis by System Gold software (Beckman Instruments, Fullerton, California). A sector mass spectrometer of the reverse geometry (i.e., magnetic sector-electric sector configuration) (Finnigan MAT, Bremen, Germany) fitted with an electrospray ionization source (Finnigan MAT, Germany) was used as the analyte detector. A 63 cm long silica separation capillary was prepared by flushing with a 50% sodium ethoxide solution in ethanol for 10 minutes using 20 p.s.i. of pressurized nitrogen gas, followed by a similar 10-minute rinse with methanol. The output end of the CE capillary was introduced directly into the electrospray source in a coaxial manner as described by A.J. Tomlinson et al, Am. Lab., 26, 29-36 (1994).

A preseparation processor as disclosed in Figure 1 was constructed using a 22 gauge stainless steel tube containing a packed volume about 0.5 mm long of PLRP-S polymer based reversed phase packing material (Polymer Labs, Amherst, Massachusetts) having a 20 micron particle size and 300Å pores. This packing material was confined by polyethylene wool frits that were placed within the stainless tube using a fine or narrow gauge wire. The microcolumn was fabricated using standard silica based, polyimide coated, capillary electrophoresis tubing with a 50 micron inside diameter. A '5-minute' epoxy (EPOXI-PATCH) was used for both sealing and electrical insulation. After allowing the epoxy to cure overnight, the sample processor was attached to the separation capillary to form an assembly using a connector consisting of a single piece of polyethylene tubing placed onto the end of the glass connecting tube. The entire assembly was preflushed with a CE separation buffer consisting of a 1% acetic acid solution (Aldrich Chemical Co., Milwaukee, WI) in water (Burdick & Jackson/Baxter, Muskegon, MI).

In the first trial, a mixture of three peptides having the amino acid sequences SIINFEKL, SIINFEKLT, and SGINFEKL, and having a net concentration of approximately 1 picomole/µl, was introduced into the assembly. These peptides were eluted from the stationary phase of the processor apparatus an elution buffer (250 nl) consisting of 80:20:1 MeOH:H_2O:TFA (TFA = trifluoroacetic acid).

In the second trial, transient isotachophoresis was added such that eluted analytes were sandwiched between an acidic and a basic buffer in an attempt to cause zone focusing and the attendant

improved resolution. An identical amount of the same peptide mixture was introduced into the same processor apparatus after thorough cleaning and equilibration as described above. Base (200 nl) consisting of 0.1% ammonium hydroxide in water was introduced prior to the elution buffer. Next, the elution buffer consisting of 80:20:1 MeOH:H_2O:TFA was used to elute the analytes, after which acid (200 nl) consisting of 1% acetic acid in water was introduced. Separation was initiated by applying about 22 kV across the ends of the assembly. A positive potential of 30 kV was applied to the inlet end of the capillary, and the outlet end of the capillary was installed into the mass spectrometer and held at a potential of about 8 kV, such that the electric field spanned the combination of the preseparation processor and separation capillary.

Results. The results of the first trial, using the simple one-step PC-CE-MS method, are shown in Figure 5. Figure 5 shows ion chromatogram of each peptide as well as a total ion current chromatogram. Zone broadening is excessive and compromises both analyte detection and the sensitivity of this assay. This result may be improved substantially by employing the analyte focusing technique of transient isotachophoresis as demonstrated in the second trial. The much improved separation resolution shown in Figure 6 is obtained.

Example 3. Use of Field Amplification in PC-CE-MS

Method. Capillary electrophoresis with a preseparation processor was used for the separation of three structurally similar peptides. Specifically, capillary electrophoresis was performed on a Beckman Instruments P/ACE 2100 capillary electrophoresis system (Fullerton, California) modified for use with electrospray mass spectrometry that was coupled to an IBM-compatible Reason Technology 486 Personal Computer (Rochester, Minnesota) using system control and data capture and analysis by system Gold software (Beckman Instruments, Fullerton, California). A sector mass spectrometer of the reverse geometry (i.e., magnetic sector-electric sector configuration) (Finnigan MAT, Bremen, Germany) fitted with an electrospray ionization source (Finnigan MAT, Germany) was used as the analyte detector. A 63 cm long silica separation capillary was prepared by flushing with a 50% sodium ethoxide solution in

ethanol for 10 minutes using 20 p.s.i. of pressurized nitrogen gas, followed by a similar 10-minute rinse with methanol. The output end of the CE capillary was introduced directly into the electrospray source in a coaxial manner as described elsewhere by A.J. Tomlinson et al. (Am. Lab., 26, 29 (1994)).

A preseparation processor as disclosed in Figure 1 was constructed using a 22 gauge stainless steel tube containing a packed volume about 0.5 mm long of PLRP-S polymer based reversed phase packing material (Polymer Labs, Amherst, Massachusetts) with a 20 micron particle size and 300 Å pores. This packing material was confined by polyethylene wool frits that were placed within the stainless tube using a fine gauge wire. The column was fabricated using standard silica based, polyimide coated, capillary electrophoresis tubing with a 50 micron inside diameter. A '5-minute' epoxy (EPOXI-PATCH) was used for both sealing and electrical insulation. After allowing the epoxy to cure overnight, the connector which consisted of a single piece of polyethylene tubing was placed onto the end of the glass connecting tube. The entire assembly was preflushed with a CE separation buffer consisting of 2 mM ammonium acetate in 1% acetic acid.

A mixture (1.4 µl) of three peptides possessing the sequences, SIINFEKL, SIINFEKLT, and SGINFEKL, prepared in separation buffer and having a net concentration of approximately 1 picomole/µl, was introduced into the preseparation processor apparatus using 20 p.s.i. of pressurized nitrogen. A small volume (63 nl) of water was introduced prior to analyte elution. Analytes were eluted from the solid phase using 80:20:1 MeOH:H_2O:TFA (125 nl), followed by the introduction of an acidic stacking buffer (125 nl) consisting of 1% acetic acid in water. Analyte focusing and subsequent separation were initiated by applying about 22 kV across the ends of the assembly. A positive potential of 30 kV was applied to the inlet end of the capillary, and the outlet end of the capillary was installed into the mass spectrometer and held at a potential of about 8 kV, such that the electric field spanned the combination of the preseparation processor and separation capillary.

Results. Ion chromatograms showing separation of the three peptide analytes utilizing the preseparation processor of the invention combined with field amplification is shown in Figure 7.

Also shown is a total current ion chromatogram. The improvement in resolution attributable to the inclusion of field amplification in separation method is evident from a comparison of the ion chromatograms shown in Figures 5 and 7. The resolution of the broad peaks observed in Figure 7 is improved slightly in Figure 6, with isotachophoresis. Thus, field amplification techniques may be used to enhance the resolution of a separation when performing PC-CE-MS using the preseparation processor of the invention.

Example 4. Improved Separation Resolution with the Preseparation Processor using a Sample Processing Membrane and Transient Isotachophoresis

Method. Capillary electrophoresis with a preseparation processor was used for the separation of a nine component mixture containing bradykinin, angiotensin II, α-melanocyte stimulating hormone, thyrotropin releasing hormone, leuteinizing hormone releasing hormone, leucine enkephalin, bombesin, methionine enkephalin, and oxytocin available from Bio-Rad Laboratories (San Jose, California). Capillary electrophoresis was performed on a Beckman Instruments P/ACE 2100 capillary electrophoresis system (Fullerton, California) modified for use with electrospray mass spectrometry that was coupled to an IBM-compatible Reason Technology 486 Personal Computer (Rochester, Minnesota) using system control and data capture and analysis by SYSTEM GOLD software (Beckman Instruments, Fullerton, California). A sector mass spectrometer of the reverse geometry (i.e., magnetic sector-electric sector configuration) (Finnigan MAT, Bremen, Germany) fitted with an electrospray ionization source (Finnigan MAT, Bremen, Germany) was used as the analyte detector. A 63 cm long silica separation capillary was prepared by flushing with a 50% sodium ethoxide solution in ethanol for 10 minutes using 20 p.s.i. of pressurized nitrogen gas, followed by a similar 10-minute rinse with methanol. The output end of the CE capillary was introduced directly into the electrospray source in a coaxial manner as described previously by A.J. Tomlinson et al. (Am. Lab., 26, 29 (1994)). Typically, a sheath liquid consisting of 60:40:1 isopropanol:H_2O:acetic acid was employed during electrospray MS.

A preseparation processor as disclosed in Figure 2 was constructed using a 22 gauge stainless steel tube containing a packed volume about 0.5 mm long of a styrene divinyl benzene based polymer membrane (Varian, San Jose, California). Frits were unnecessary because the membrane is adequately confined by the connecting tubes illustrated in Figure 2 and discussed in related text. The column was fabricated using standard silica based, polyimide coated, capillary electrophoresis tubing with a 50 micron inside diameter, with a '5-minute' epoxy (EPOXI-PATCH) for both sealing and electrical insulation. Two versions of the microcolumn were fabricated in this work, and each produced identical results. This first consisted of a microcolumn totally encased and sealed in epoxy. In the second, the epoxy was used only to seal the connecting tubes in place, and a combination of this epoxy, air, and the teflon housing of the PAC/E CE unit was used to provide electrical insulation to the processing microcolumn. After allowing the epoxy to cure overnight, a connector consisting of a single piece of polyethylene tubing was placed onto the end of the glass connecting tube. The entire assembly was cleaned by flushing with MeOH and preflushed with a CE separation buffer consisting of a solution containing 2 mM NH_4OH: 1% acetic acid solution in water (Burdick & Jackson/Baxter) for approximately 10 minutes.

The peptide mixture (1.4 µl), prepared in separation buffer and having a total protein concentration of approximately 1 picomole/µl, was introduced into the membrane based processor assembly using pressure injection at 20 p.s.i. for 1 minute. Approximately 1.4 picomoles of each peptide was introduced into the apparatus. The analytes were adsorbed onto the solid phase, and were rinsed with run buffer for 3 minutes using standard pressure injection using 20 p.s.i. of pressurized nitrogen gas. As in Example 2, three liquid buffer solutions were used to prepare the system for transient isotachophoresis. First, base (112 nl) consisting of 0.1% NH_4OH in water was introduced into the PC-CE-MS assembly. Next, the peptides were eluted from the membrane-based stationary phase of the processor apparatus using an elution buffer (112 nl) consisting of 80:20:1 MeOH:H_2O:TFA. Elution was followed by the injection of acid (56 nl) consisting of 1% acetic acid in water. Separation and isotachophoresis were initiated by applying a potential difference of

about 22 kV across the ends of the assembly. A positive potential of 30 kV was applied to the inlet end of the capillary, and the outlet end of the capillary was installed into the mass spectrometer and held at a potential of about 8 kV, such that the electric field spanned the combination of the preseparation processor and separation capillary.

Results. Figure 8 shows the ion chromatogram of each analyte using transient isotachophoresis with a membrane-based PC-CE-MS microcolumn. The advantages of using membrane-based versus particulate-based solid phase packing materials in the preseparation processor can be seen by comparing the data in Figures 5, 6, and 8. Use of a membrane results in a dead volume that is much smaller compared to that produced using the more familiar particulate solid phase materials. This reduction in dead volume allows a reduction in the volume of the elution buffer used. These conditions lead to a marked improvement in the resolution observed in the separation. Thus, analyte focusing via transient isotachophoresis may be used successfully with a preseparation processor assembly that contains a membrane-based solid phase processing material.

Example 5. Larger Volumes of Leading and Trailing Buffers Improve Separation Resolution with the Preseparation Processor using a Sample Processing Membrane and Transient Isotachophoresis

Method. Capillary electrophoresis with a preseparation processor was used to separate a nine-component peptide mixture containing bradykinin, angiotensin II, α-melanocyte stimulating hormone, thyrotropin releasing hormone, leuteinizing hormone, releasing hormone, leucine enkephalin, bombesin, methionine enkephalin, and oxytocin available from Bio-Rad Laboratories (San Jose, California). Capillary electrophoresis was performed on a Beckman Instruments P/ACE 2100 capillary electrophoresis system (Fullerton, California) modified for use with electrospray mass spectrometry that was coupled to an IBM compatible Reason Technology 486 Personal Computer (Rochester, Minnesota) using system control and data capture and analysis by SYSTEM GOLD software (Beckman Instruments, Fullerton, California). A sector mass spectrometer of the reverse geometry (i.e., magnetic sector-electric

sector configuration) (Finnigan MAT, Bremen, Germany) fitted with an electrospray ionization source (Finnigan MAT, Germany) was used as the analyte detector. A 63 cm long silica separation capillary was prepared by flushing with a 50% sodium ethoxide solution in ethanol for 10 minutes using 20 p.s.i. of pressurized nitrogen gas, followed by as a similar 10-minute rinse with methanol. The output end of the CE capillary was introduced directly into the electrospray source in a coaxial manner as described previously by A.J. Tomlinson et al. (Am. Lab., 26, 29 (1994)). Typically, a sheath liquid consisting of 60:40:1 isopropanol:H_2O:acetic acid was employed during electrospray MS.

A preseparation processor as disclosed in Figure 2 was constructed using a 22 gauge stainless steel tube containing a packed volume about 0.5 mm long of a styrene divinyl benzene based polymer membrane (Varian, San Jose, California). Frits were unnecessary because the membrane is adequately confined by the connecting tubes illustrated in Figure 2 and discussed in accompanying text. The microcolumn was fabricated using standard silica based, polyimide coated, capillary electrophoresis tubing with a 50 micron inside diameter, and a '5-minute' epoxy (Epoxi-Patch) for both sealing and electrical insulation. Two versions of the microcolumn were fabricated in this work, and each produced identical results. This first consisted of a microcolumn totally encased and sealed in epoxy. In the second, the epoxy was used only to seal the connecting tubes in place, and a combination of this epoxy, air, and the teflon housing of the PAC/E CE unit were used to provide electrical insulation to the processing microcolumn. After allowing the epoxy to cure overnight, a connector consisting of a single piece of polyethylene tubing was placed onto the end of the glass connecting tube. The entire assembly was cleaned by flushing with MeOH and preflushed with a CE separation buffer consisting of a solution containing 2 mm NH_4OH: 1% acetic acid solution in water (Burdick & Jackson/Baxter) for approximately 10 minutes.

The peptide mixture (1.4 µl) prepared in separation buffer and having a total protein concentration of approximately 1 picomole/µl was introduced into the membrane-based processor assembly using pressure injection at 20 p.s.i. for 1 minute. Approximately 1.4 picomoles of each peptide was introduced into the

apparatus. Analytes were adsorbed onto the solid phase, and were rinsed with run buffer for 3 minutes using standard pressure injection using 20 p.s.i. of pressurized nitrogen gas. As in Examples 2 and 4, three liquid buffer solutions were used to prepare the system for transient isotachophoresis. First, a base (210 nl) consisting of 0.1% NH_4OH in water was introduced into the PC-CE-MS assembly using 20 p.s.i. of pressurized nitrogen for 0.15 minutes. Next, the peptides were eluted from the membrane-based stationary phase of the processor apparatus by injecting a buffer (238 nl) consisting of 80:20:1 MeOH:H_2O:TFA using 20 p.s.i. of nitrogen for 0.17 minutes. Then acid (70 nl) consisting of 1% acetic acid in water was injected using 20 p.s.i. of nitrogen for 0.05 minutes. Separation and isotachophoresis were initiated by applying a potential difference of about 22 kV across the ends of the assembly. A positive potential of 30 kV was applied to the inlet end of the capillary, and the outlet end of the capillary was installed into the mass spectrometer and held at a potential of about 8 kV, such that the electric field spanned the combination of the preseparation processor and separation capillary, and by applying 0.5 p.s.i. of nitrogen gas in the forward direction.

Results. Figure 9 shows the ion chromatogram of each analyte component of peptide mixture containing large amounts of analyte, using a CE separation method utilizing transient isotachophoresis and a membrane-based PC-CE-MS microcolumn. Comparison of the data in Figures 8 and 9 shows that enhanced analyte resolution (Figure 9) can be effected by increasing volumes of leading, trailing and elution buffers. However, this is at the expense of increased analysis times.

Example 6. Processing of Urine Samples Using a Preseparation Processor Containing a Sample Processing Membrane

Method. A capillary electrophoresis system and processor apparatus similar to that used in Example 4 and 5 was used in this experiment to analyze the drug metabolites excreted in patient urine after the administration of the neuroleptic drug Haloperidol. Capillary electrophoresis was performed on a Beckman Instruments P/ACE 2100 capillary electrophoresis system (Fullerton, CA) modified for use with electrospray mass spectrometry that was coupled to an

IBM compatible Reason Technology 486 Personal Computer (Rochester, MN) using system control and data capture and analysis by System Gold™ software (Beckman Instruments, Fullerton, CA). A sector mass spectrometer of the forward geometry (i.e., electric sector-magnetic sector configuration) (Finnigan MAT, Bremen, Germany) fitted with an electrospray ionization source (Analytica, Banford, CT) was used for analysis. The metabolites were detected using state-of-the-art position and time resolved ion counting (PATRIC™) detector (Finnigan MAT, Bremen, Germany). An electrospray voltage of -3.4 kV and an instrument resolution of 1000 were used in this work. The scan range was set to 150-450 Da at 5 seconds per decade, and an 8% mass window was employed during PATRIC™ detection.

An 80 cm long silica separation capillary was prepared by flushing with a 50% sodium ethoxide solution in ethanol for 10 minutes using 20 p.s.i. of pressurized nitrogen gas, followed by similar 10 minute rinse with methanol. The output end of the CE capillary was introduced directly into the electrospray source in a coaxial manner as described previously by A.J. Tomlinson et al, American Lab., 26, 29-36 (1994). Typically, a sheath liquid consisting of 60:40:1 isopropanol:H_2O:acetic acid was employed during electrospray MS.

A preseparation processor as disclosed in Figure 2 was constructed using a 22 gauge stainless steel tube containing a packed volume about 0.5 mm long of EMPORE™ (Varian, San Jose, CA) membrane containing silica particles derivatized with C_{18} alkyl groups and confined by a membrane. Frits were unnecessary in this unit because the membrane and solid phase are adequately confined by the connecting tubes illustrated in Figure 2 and discussed above. The microcolumn was fabricated using standard silica based, polyimide coated, capillary electrophoresis tubing with a 50 micron inside diameter, and a '5-minute' epoxy (EPOXI-PATCH) for both sealing and electrical insulation. Two versions of the microcolumn were fabricated in this work, and each produced identical results. The first consisted of a microcolumn totally encased and sealed in epoxy. In the second, the epoxy was used only to seal the connecting tubes in place, and the necessary electrical insulation was provided by a combination of the epoxy seal, air, and the teflon housing of the PAC/E CE unit. After allowing the epoxy to cure overnight, the

sample processor was attached to the separation capillary using a connector consisting of a single piece of polyethylene tubing placed onto the end of the glass connecting tube. The entire assembly was cleaned by flushing with MeOH and preflushed with a CE separation buffer consisting of a solution containing a 50 mM NH_4OAc:10% MeOH:1% acetic acid solution in water (Burdick & Jackson/Baxter) for approximately 10 minutes.

A sufficient volume of urine was collected from a female patient receiving a dosage 0.5 mg of Haloperidol/day. The urine was treated with zinc sulfate and centrifuged to remove the protein. Approximately 10 µl of the supernatant was introduced into the processor apparatus. The analytes were adsorbed onto the solid phase and rinsed with separation buffer for 3 minutes. This rinsing was performed via standard pressure injection using 20 p.s.i. pressurized nitrogen gas in order to remove urea, salts, and other undesirable components from the solid phase. Next, the peptides were eluted from the membrane-based stationary phase of the processor apparatus by injecting a buffer consisting of 100% MeOH using 20 p.s.i. of nitrogen for 0.08 minutes. The peptides were then pushed into the CE separation capillary by a small plug of separation buffer which was injected by applying 20 p.s.i. of nitrogen gas for 0.1 min. Separation and analysis was initiated by applying 20 kV across the ends of the assembly with a positive potential applied to the inlet end of the assembly and a negative potential applied to the outlet end of the assembly, such that the electric field spanned the combination of the preseparation processor and separation capillary.

Results. Figure 10 shows the ion chromatograms of the various drug metabolites. In the absence of the sample processor, the electrospray ionization scanner becomes clogged with urea and ceases to function. Thus, an apparatus and method of analysis are demonstrated which utilize a separation buffer containing a substantial fraction of organic solvent and that successfully remove contaminants from biological samples.

Example 7. Comparison of Conductive and Non-Conductive Preconcentration Cartridges Using an Adsorptive Membrane.

Method. All experiments were performed using a Beckman P/ACE 2100 instrument (Fullerton, CA, USA) coupled to a Reason Technology 486 PC (Rochester, MN, USA) with system control and data capture by System Gold software (Beckman), as described in previous examples. Dimethylformamide (DMF) detection was by UV at a wavelength of 214 nm.

The preconcentration capillary used in these experiments was prepared from uncoated fused silica tubing (50 µm i.d.), pretreated with potassium methoxide, methanol, and finally CE separation buffer (1% acetic acid in water). The final dimension of the PC-CE capillary was 67 cm x 50 µm i.d. A piece of polymeric (styrene divinyl benzene) membrane was installed at the mid-point of either a teflon or stainless steel cartridge. A fused silica capillary was set into each end of the cartridge with solvent resistant epoxy resin. Prior to installation, the membrane was activated by washing with MeOH, then CE separation buffer. The entire PC-CE capillary was then conditioned under high pressure (20 p.s.i.) for ten minutes with CE separation buffer (1% acetic acid in water). The flow rate through the PC-CE capillary was measured (using a CE separation buffer rinse under high pressure) to ensure that final results could be normalized and compared.

Measurement of EOF in PC-CE capillaries was determined by the migration of dimethylformamide (DMF). This compound is neutral under CE conditions and hence is mobilized by EOF only under CE conditions. A variety of PC-CE cartridge capillaries were made involving a combination of metal, Teflon, one membrane or two membranes, and C-18 solid phase, as summarized in Table 1. The neutral marker dimethylformamide (DMF) was pressure injected via the outlet onto the PC-CE capillary. This ensured that the membrane or solid phase did not come into contact with DMF. The DMF was then pushed into the capillary with CE separation medium (1% acetic acid) under high pressure reverse rinse (0.45 minutes, ~800 nL). Migration of DMF was determined under two conditions: 1) low pressure (0.5 p.s.i.) infusion; and 2) simultaneous application of 30 kV and low pressure infusion using a

CE separation medium of 1% acetic acid in water. Between analyses, the PC-CE cartridge and capillary were rinsed with CE separation medium. These experiments were carried out on virgin membranes or C-18 solid phase and after the adsorptive phase has been contaminated with 1-2 picomoles of a peptide mixture consisting of 5 components OVA, OVAG, OVA9, VSV and P_2Cl. Each experiment was conducted in triplicate and all the results are summarized in Table 1. As summarized in Table 1, in all cases, after peptide had passed through the adsorptive phase migration of the DMF marker slowed. Furthermore, whether the membrane/solid phase was encased in either Teflon or metal, the percent slowing was approximately the same. This is summarized in Table 2. With the exception of solid phase in the metal cartridge, all other slowing rates were comparable (~80-90%). In particular the use of 2 membranes in concert (designed to mimic solid phase experiments since adsorptive bed is approximately the same for 2 membranes and 3 mm solid phase), showed no distinguishable difference for metal or teflon as the cartridge. Comparison of the slowing of DMF migration in teflon versus metal (Table 3) is simply a function of flow rate (Table 1 and Table 4) through the adsorptive phase.

Example 8. Use of Analyte Stacking in PC-CE-MS.

The capillary electrophoresis system described in Example 3, in conjunction with analyte stacking, was used to separate a mixture of the neuroleptic drug haloperidol (HAL) and six putative metabolites (HAL, CPHP, HNO, HP+, HTP, HTPNO and RHAL.

A preseparation processor as disclosed in Figure 2 was constructed from uncoated fused silica tubing, pretreated with potassium methoxide, methanol, and finally CE separation buffer. A piece of polymeric (SDB) membrane was installed at the mid-point of a Teflon cartridge. A fused silica capillary was set into each end of the cartridge with solvent-resistant epoxy resin. Prior to installation, the membrane was activated by washing with MeOH, then CE separation buffer. The entire mPC-CE capillary was then conditioned under high pressure (20 p.s.i.) for ten minutes with CE separation buffer. All subsequent capillary treatments and sample loading, washing, and elution were also carried out under high pressure (20 p.s.i.).

Separation was performed on a Beckman P/ACE 2100 instrument using a purpose built mPC-CE capillary. The sample (~380 attomoles/nL) was loaded in separation buffer by pressure injection for one minute. The membrane was then washed with separation buffer (5 μL) consisting of 50 mM NH_4OAc/10% MeOH/1% AcOH and finally the mixture was eluted with 1:1 MeOH: CH_3CN (50 nL). Separation buffer consisted of 50 mM NH_4OAc, 10% MeOH, and 1% AcOH. Separation was affected at 30 kV (25 μA), with the capillary maintained at room temperature and monitored at 214 nm.

All seven components were baseline resolved and resolution and peak width were comparable to CE analyses and were much improved over PC-CE analysis using solid phase packing material. It is interesting to note that the relatively more hydrophilic compound, CPHP, did not adhere as well to the membrane as other compounds and may have been lost during the wash step.

TABLE 1 Normalized DMF Migration in PC-CE Capillaries

PC Cartridge	No. of SDB membrane	Flow Rate in PC-CE capillary (μL/min)	Peptide Contamination	Current (μA)	DMF Migration Low Pressure (nL/min)	Average LP only	DMF migration 30 kV + LP (nL/min)	Average 30 kV + LP
Metal	1	1.7	No	6.5	49.3, 49.3, 48.7	49.1	98.6, 101.1, 103.7	101.1
Metal	1	—	Yes	6.5	47.8, 49.0, 47.6	48.1	49.6, 77.6, 82.4	80.0*
Teflon	1	1.6	No	6.3	46.1, 47.6, 45.3	46.3	67.7, 81.7, 82.6	77.3
Teflon	1	—	Yes	6.3	45.9, 46.4, 46.1	46.1	62.1, 71.6, 72.3	68.7
Metal	2	1.4	No	6.4	49.4, 47.8, 47.8	48.3	98.0, 113.1, 113.1	108.1
Metal	2	—	Yes	6.4	46.3, 44.9, 48.2	46.5	94.8, 98.0, 93.3	95.4
Teflon	2	1.8	No	6.5	56.4, 56.0, 54.4	55.6	126.0, 128.1, 128.1	127.4
Teflon	2	—	Yes	6.5	53.6, 52.5, 52.1	52.7	105.0, 114.5, 112.8	110.8
Teflon	3 mm C-18 solid	1.3	No	6.5	41.7, 41.4, 40.4	41.2	97.5, 97.5, 95.8	96.9
Teflon	3 mm C-18 solid	—	Yes	6.5	42.3, 41.7, 41.1	41.7	80.3, 88.1, 91.0	86.5
Metal	3 mm C-18 solid	2.0	No		59.2, 58.8	59.0	40.0, —, —	40.0
Metal	3 mm C-18 solid	—	Yes		56.8, 57.7	57.2	15.0, —, —	15.0

TABLE 2

Percent Slowing of DMF Migration (under LP + 30 kV) After Contamination with Peptide

PC Cartridge	No. of Membranes	% Slowing of DMF Migration
Metal Cartridge	1	79.1
Teflon Cartridge	1	88.9
Metal Cartridge	2	88.3
Teflon Cartridge	2	86.9
Teflon	3 mm Solid Phase C-18	89.3
Metal	3 mm Solid Phase C-18	37.5*

*Based on only a single measurement - since could not obtain any data after 1st run - hence this data point to be regarded with caution.

TABLE 3

Comparison of DMF Migration in Teflon Versus Metal

No of Membranes	Contamination with Peptide	% Teflon DMF/Metal DMF
1	No	76.5
1	Yes	85.8
2	No	117.9
2	Yes	116.1

TABLE 4

Comparion of Flow Rate in PC-CE

Comparison	Peptide Comtamination	Membrane	Percent
Teflon/Metal	No	1	94.1
Teflon/Metal	No	2	128.6

Example 9. Direct Analysis of Aqueous Humor by Membrane Preconcentration Matrix-Assisted Laser Desorption Time of Flight Mass Spectrometry (MALDI-TOF-MS)

Method. A sample preprocessor (Figure 12) was constructed by inserting an EMPORE™ styrene divinyl benzene copolymer membrane into a Teflon housing, then inserting short (1.5 cm) lengths of 50 µm i.d. fused silica capillary tubing into each end of the housing to physically confine and permit liquid flow through the membrane. The housing was sealed onto the capillary tubing using a solvent resistant epoxy resin to prevent leakage and to provide mechanical strength. The capillary tubing forming the inlet end of the sample preprocessor was connected to a nitrogen pressure bomb, and the tubing forming the outlet end of the sample preprocessor was connected to another length of capillary tubing by means of a polyethylene sleeve throughout the entire experiment. This length of capillary tubing was in turn connected to a Bruker Biflex time of flight mass spectrometer equipped with both linear and relectron detection systems (Bruker Instruments, Billerica, MA).

All preprocessor activation, conditioning, washing, sample loading and analyte elution was performed by connecting this device to the outlet of the pressurized bomb and applying 40 p.s.i. of pressure. The preprocessor was activated with methanol (150 µl), followed by 2 mM ammonium acetate:1% acetic acid in water (150 µl). Aqueous humor obtained by microcatheter from a human eye (5 - 10 µl) was applied, without pretreatment, to the activated preprocessor. The preprocessor was washed with 2 mM ammonium acetate:1% acetic acid in water (150 µl) to remove salts and other hydrophilic matrix components. Analytes were then eluted from the preprocessor with methanol (~5 µl). A portion of the eluate (1-2 µl) was spotted onto a MALDI-TOF-MS target that had been prespotted with 2 µl matrix (matrix: a saturated solution of α-cyano-4-hydroxy cinnamic acid (Sigma Chemical Co., St. Louis, MO) in acetonitrile:water:trifluoroacetic acid 70:30:0.1 v/v/v). The time of flight mass spectrometer was operated in the linear mode, and an accelerating voltage of 25 kV was used.

Results. Figure 14 shows MALDI-TOF-MS spectrum of the aqueous humor sample. A number of protein responses were observed, including human serum albumin (MH^+ = 66639.6, MH_2^{2+}=33370). In control experiments where aqueous humor is spotted onto a MALDI-TOF-MS target without preprocessing, no protein responses were detected. It is likely that the high salt content of aqueous humor interferes with the crystallization and ionization processes necessary for MALDI-TOF-MS analysis. Use of the sample preprocessor permits sample cleanup by desalting and thus facilitates protein analysis of this complex, physiologically derived fluid using MALDI-TOF-MS.

The complete disclosures of all patents, patent documents, and publications, are incorporated herein by reference as if individually incorporated. It will be appreciated by those skilled in the art that various modifications can be made to the above described embodiments of the invention without departing from the essential nature thereof. The invention is intended to encompass all such modifications within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A sample preprocessor comprising a container comprising a first port, a second port, and an interior space partitioned by at least one membrane into a first compartment comprising the first port and a second compartment comprising the second port, so that a liquid sample that enters the interior space through the first port and exits the interior space through the second port traverses the membrane.

2. The sample preprocessor of claim 1 wherein the container is tubular and each of the first and second compartments is cylindrical.

3. The sample preprocessor of claim 2 wherein the container is a microbore capillary electrophoresis tube.

4. The sample preprocessor of claim 1 wherein the container is made of polytetrafluoroethylene.

5. The sample preprocessor of claim 1 wherein the membrane comprises an organic polymer.

6. The sample preprocessor of claim 5 wherein the membrane comprises a chemically inert organic polymer matrix embedded with adsorptive particles.

7. The sample preprocessor of claim 1 wherein the membrane comprises an adsorptive material for concentrating an analyte of a liquid sample comprising at least one analyte.

8. The sample preprocessor of claim 1 wherein the membrane comprises a molecular weight filter.

9. The sample preprocessor of claim 1 wherein a plurality of membranes partitions the interior space into at least two compartments so that a liquid that enters the interior space through the first port and exits the interior space through the second port traverses each of the membranes.

10. The sample preprocessor of claim 9 wherein at least one of the plurality of membranes is a chemically inert organic polymer matrix embedded with adsorptive particles.

11. The sample preprocessor of claim 9 wherein at least one of the plurality of membranes is a molecular weight filter.

12. The sample preprocessor of claim 1 further comprising confinement means for retaining the at least one membrane inside the container.

13. A sample preprocessor assembly comprising:
(a) the sample preprocessor of claim 1,
(b) sample introduction means in fluid communication with the first port to form an inlet connection;
(c) sample removal means in fluid communication with the second port to form an outlet connection; and
(d) sealant for sealing each of the inlet connection and the outlet connection to prevent fluid leakage from each connection when in use.

14. The sample preprocessor assembly of claim 13 wherein the membrane comprises a chemically inert organic polymer matrix embedded with adsorbent particles.

15. The sample preprocessor assembly of claim 13 wherein the membrane comprises an adsorptive material for concentrating an analyte of a liquid sample comprising at least one analyte.

16. The sample preprocessor of claim 13 wherein a plurality of membranes partitions the interior space into at least two compartments so that a liquid that enters the interior space

through the first port and exits the interior space through the second port traverses each of the membranes.

17. The sample preprocessor assembly of claim 13 wherein the sample introduction means and the sample removal means each comprises a microbore capillary electrophoresis tube.

18. The sample preprocessor assembly of claim 13 wherein the sealant comprises a chemically resistant organic polymer.

19. The sample preprocessor of claim 18 wherein the sealant comprises a curable epoxy resin.

20. The sample preprocessor assembly of claim 13 further comprising connection means for placing the sample removal means in fluid communication with a capillary.

21. A preprocessing capillary electrophoresis system comprising:
(a) a separation capillary having an inlet end; and
(b) at least one sample preprocessor assembly of claim 13, wherein the sample removal means of the at least one sample preprocessor assembly is in fluid communication with the inlet end of the separation capillary.

22. The preprocessing capillary electrophoresis system of claim 21 comprising a plurality of sample preprocessor assemblies comprising a first sample preprocessor assembly and a last sample preprocessor assembly, connected in series in fluid communication with each other and with the separation capillary so that a liquid that enters the interior space of the first sample preprocessor assembly through the first port of the first sample preprocessor assembly and exits the interior space of the last sample preprocessor assembly through the second port of the last sample preprocessor assembly traverses the at least one membrane each of the sample preprocessor assemblies prior to entering the inlet end of the separation capillary.

23. The preprocessing capillary electrophoresis system of claim 21 further comprising electrical means for electrokinetically separating analytes present in the liquid.

24. The preprocessing capillary electrophoresis system of claim 21 wherein the membrane comprises an adsorptive material for concentrating an analyte of a liquid sample comprising at least one analyte.

24. The preprocessing capillary electrophoresis system of claim 21 wherein the membrane comprises a chemically inert organic polymer matrix embedded with adsorbent particles.

25. The preprocessing capillary electrophoresis system of claim 21 wherein a plurality of membranes partitions the interior space into at least two compartments so that the liquid sample that enters the interior space through the first port and exits the interior space through the second port traverses each of the membranes.

26. The preprocessing capillary electrophoresis system of claim 21 wherein the sample removal means is connected to the inlet end of the separation capillary by microbore capillary electrophoresis tubing internally coated with silanizing reagents or hexadimethrine bromide.

27. A method for performing preprocessing capillary electrophoresis comprising:
(a) contacting a liquid sample comprising a plurality of analytes with the at least one membrane of a preprocessing capillary electrophoresis system of claim 21 to yield at least one preprocessed analyte;
(b) separating the preprocessed analyte by introducing at least one electrically conductive buffer into the preprocessing capillary electrophoresis system and applying an electric field across the preprocessing capillary electrophoresis system to yield a separated analyte;
(c) focusing the separated analyte; and

(d) detecting the separated analyte.

28. The method of claim 27 wherein transient isotachophoresis is used to focus the separated analyte.

29. The method of claim 27 wherein the at least one membrane comprises an adsorptive material for concentrating an analyte, and wherein the preprocessed analyte of step (a) comprises a concentrated analyte adsorbed onto said membrane, said method further comprising an elution step performed prior to step (b) comprising eluting the concentrated analyte from the container by supplying at least one elution buffer that releases the concentrated analyte from the adsorptive material.

30. The method of claim 27 wherein the at least one membrane comprises a chemically inert organic polymer matrix embedded with adsorbent particles, and wherein the preprocessed analyte of step (a) comprises a concentrated analyte adsorbed onto said membrane, said method further comprising an elution step performed prior to step (c) comprising eluting the concentrated analyte from the container by supplying at least one elution buffer that releases the concentrated analyte from the membrane.

31. A method for performing preconcentration capillary electrophoresis comprising:

(a) filling the preprocessing capillary electrophoresis system of claim 21 with an electrically conductive buffer, wherein the at least one membrane comprises an adsorptive material that concentrates an analyte;

(b) introducing a liquid sample comprising a plurality of analytes into the first port of the container such that at least one analyte adsorbs onto the membrane to yield a concentrated analyte;

(c) introducing a alkaline plug comprising a base into the first port of the container;

(d) eluting the concentrated analyte from the container by supplying at least one elution buffer that releases the concentrated analyte from the adsorptive membrane;
(e) introducing immediately thereafter an acid plug comprising an acid into the first port of the container;
(f) separating the concentrated analyte by introducing at least one electrically conductive buffer into the first port of the container and applying an electric field across the preprocessing capillary electrophoresis system to yield a separated analyte; and
(h) detecting the separated analyte.

32. The method of claim 31 wherein the base in the alkaline plug and the acid in the acid plug is volatile.

33. The method of claim 31 wherein the alkaline plug is about 0.01% to 0.5% aqueous ammonium hydroxide, and the acid plug is about 0.1% to 5% aqueous acetic acid.

34. The method of claim 31 wherein the at least one membrane of the preprocessing capillary electrophoresis system comprises a chemically inert organic polymer matrix embedded with adsorbent particles.

35. A method for off-line sample preprocessing comprising contacting the liquid sample comprising a plurality of analytes with the at least one membrane of a sample preprocessor of claim 1 to yield at least one preprocessed analyte.

36. The method of claim 35 wherein the at least one membrane comprises an adsorptive material for concentrating an analyte, and wherein the preprocessed analyte comprises a concentrated analyte adsorbed onto said membrane.

37. The method of claim 36 further comprising eluting the concentrated analyte from the container by supplying at least one elution buffer that releases the concentrated analyte from the membrane.

38. A method for performing preconcentration capillary electrophoresis of multiple samples preconcentrated off-line comprising:

(a) contacting a liquid sample comprising a plurality of analytes with the at least one membrane of a first sample preprocessor assembly of claim 13, wherein the at least one membrane comprises an adsorptive material for concentrating an analyte, such that at least one analyte adsorbs onto the membrane to yield a first concentrated analyte;

(b) connecting sample removal means of the first sample preprocessor assembly to the inlet end of a separation capillary of a capillary electrophoresis system;

(c) separating the first concentrated analyte by introducing at least one electrically conductive buffer into the capillary electrophoresis system and applying an electric field across the capillary electrophoresis system to yield a first separated analyte;

(d) concurrent with step (c), contacting a liquid sample comprising a plurality of analytes with the at least one membrane of a second sample preprocessor assembly of claim 13, wherein the at least one membrane comprises an adsorptive material for concentrating an analyte, such that at least one analyte adsorbs onto the membrane to yield a second concentrated analyte;

(e) detecting the first separated analyte;

(f) disconnecting the sample removal means of the first sample preprocessor assembly from the inlet end of the separation capillary;

(g) connecting sample removal means of the second sample preprocessor assembly to the inlet end of the separation capillary of the capillary electrophoresis system;

(h) separating the second concentrated analyte by introducing at least one electrically conductive buffer into the capillary electrophoresis system and applying an electric field across the capillary electrophoresis system to yield a second separated analyte;

(i) concurrent with step (h), repeating step (a);

(j) detecting the second separated analyte; and
(k) repeating steps (b) through (j).

39. The method of claim 38 further comprising a first elution step performed immediately preceding step (c) comprising eluting the first concentrated analyte from the container of the first sample preprocessor assembly by supplying at least one elution buffer that releases the first concentrated analyte from the adsorptive material, and a second elution step performed immediately preceding step (h) comprising eluting the second concentrated analyte from the container of the second sample preprocessor assembly by supplying at least one elution buffer that releases the second concentrated analyte from the adsorptive material.

40. The method of claim 38 further comprising a first analyte focusing step performed immediately preceding step (e) comprising focusing the first separated analyte, and a second focusing step performed immediately preceding step (j) comprising focusing the second separated analyte.

41. A sample preprocessing system comprising:
(a) a capillary comprising an inlet end; and
(b) at least one sample preprocessor assembly of claim 13 wherein the sample removal means is in fluid communication with the inlet end of the capillary.

42. The sample preprocessing system of claim 41 further comprising (c) a sample processing device.

43. The sample preprocessing system of claim 42 wherein the sample processing device is selected from the group consisting of a capillary electrophoresis system, a high performance liquid chromatography (HPLC) system, a capillary HPLC system, a capillary electrochromatography system, and microdialysis system.

44. The sample preprocessing system of claim 42 further comprising (d) a sample detection device.

45. The sample preprocessing system of claim 44 wherein the sample detection device is a mass spectrometer.

46. The sample preprocessing system of claim 45 wherein the mass spectrometer is an electrospray ionization-mass spectrometer (ESI-MS) or a matrix assisted laser desorption time of flight mass spectrometer.

47. The sample preprocessing system of claim 46 wherein the electrospray ionization-mass spectrometer (ESI-MS) is a microspray ESI-MS or a nanospray ESI-MS.

48. A method for performing sample preprocessing comprising:
(a) contacting the liquid sample comprising a plurality of analytes with the at least one membrane of a sample preprocessing system of claim 44 to yield at least one preprocessed analyte;
(b) moving the preprocessed analyte from the container to the capillary;
(c) processing the preprocessed analyte to yield a processed analyte; and
(d) detecting the processed analyte.

49. The method of claim 48 wherein the processing step comprises electrophoretically or chromatographically separating the preprocessed analyte.

50. The method of claim 48 wherein the processing step comprises microdialysis.

51. The method of claim 48 wherein the detecting step comprises detecting the processed analyte by mass spectrometry.

52. The method of claim 51 wherein the mass spectrometry is carried out using an electrospray ionization-mass spectrometer

(ESI-MS) or a matrix assisted laser desorption time of flight mass spectrometer.

FIGURE 1

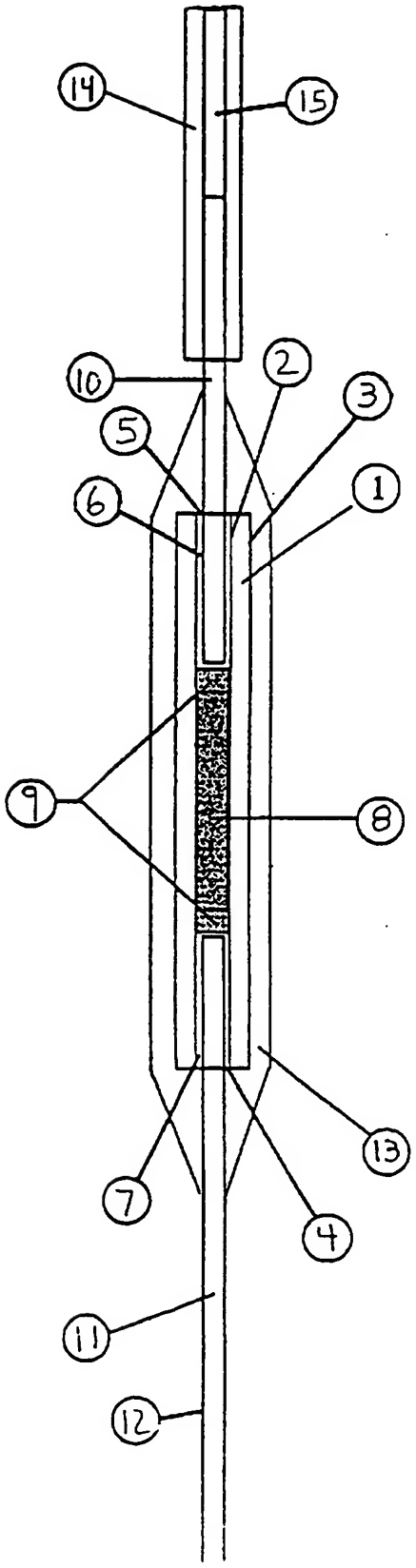
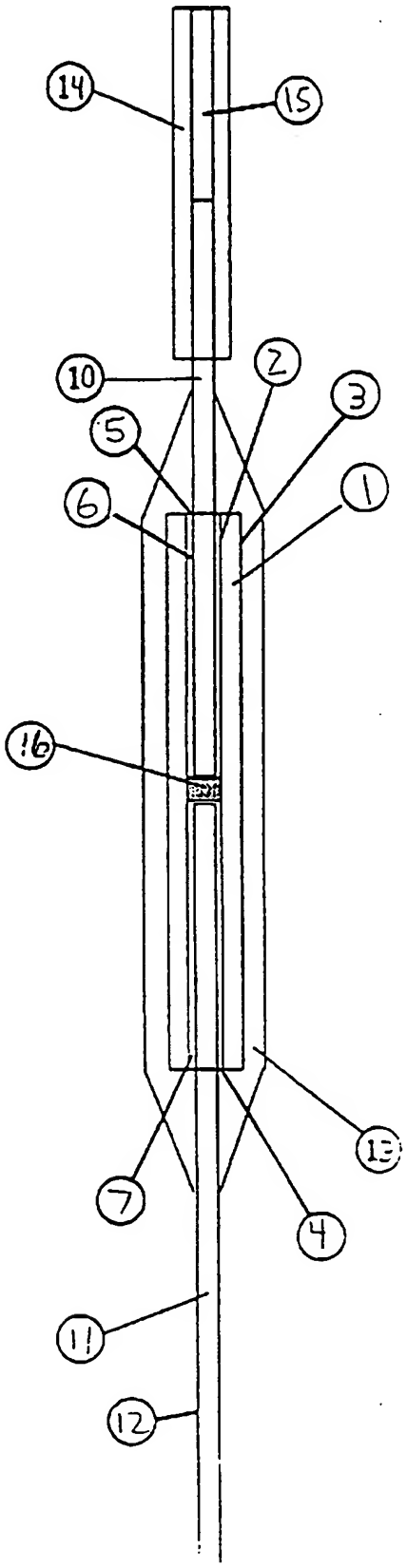


FIGURE 2



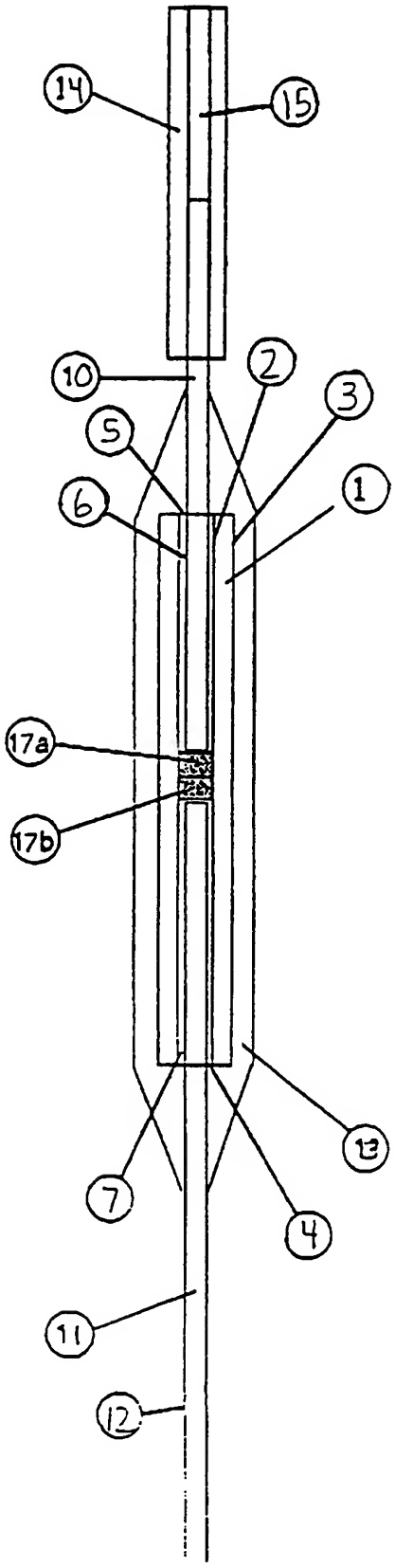


FIGURE 3

FIGURE 4

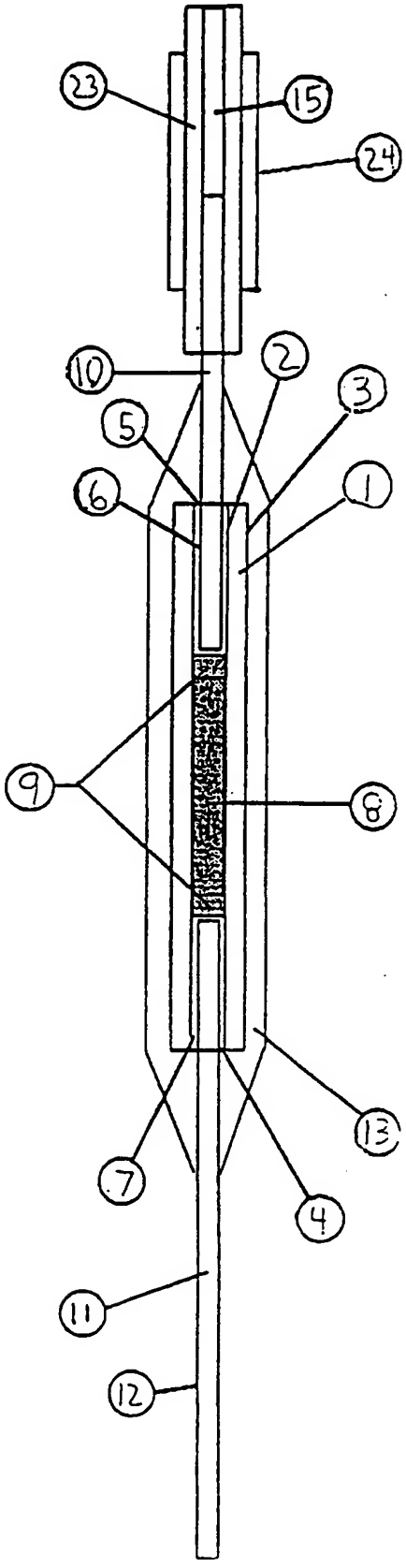
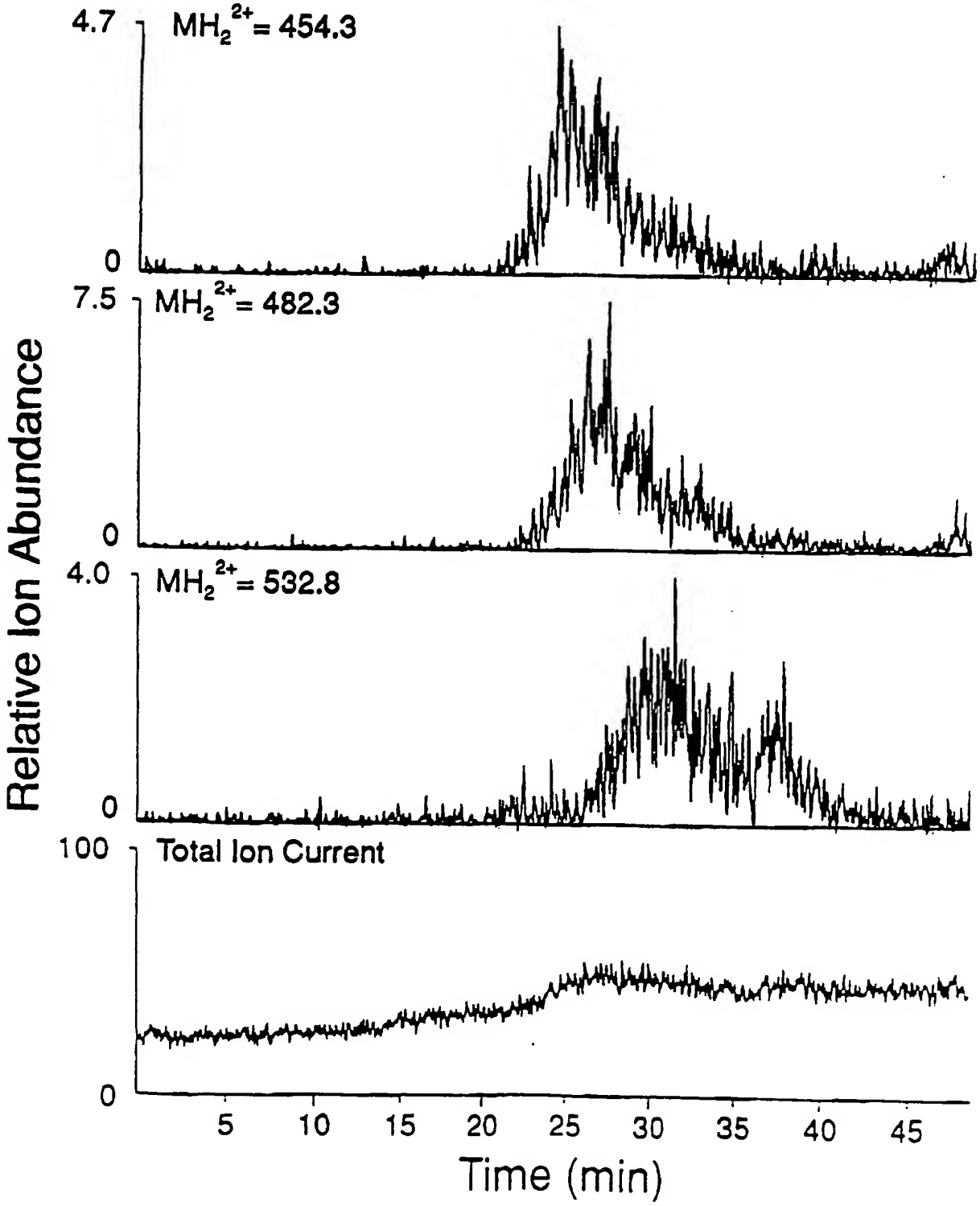


FIGURE 5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7.5
MH_2^{2+}= 482.3
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4.0
MH_2^{2+}= 532.8
0
100
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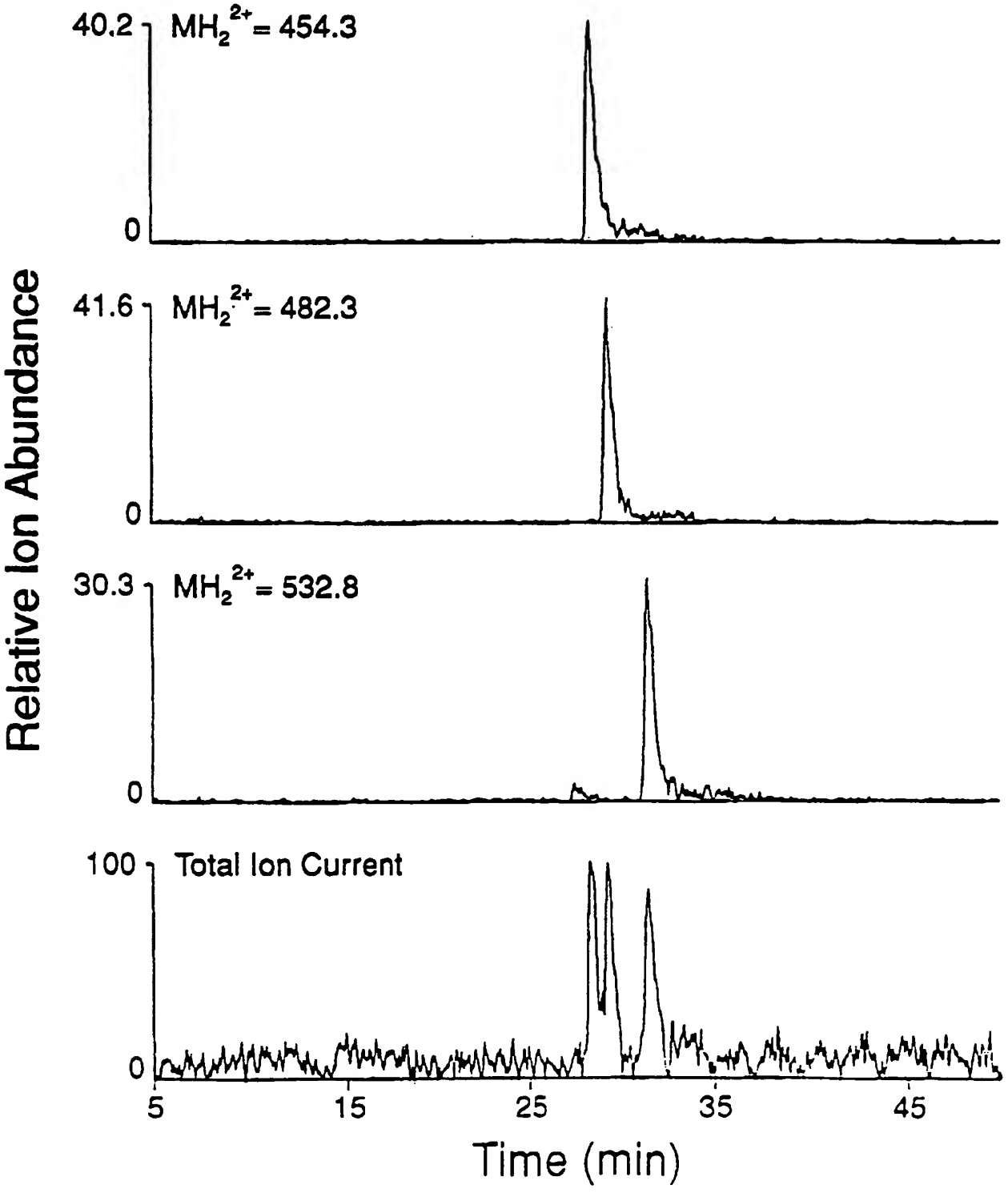
Relative Ion Abundance
5
10
15
20
25
30
35
40
45
Time (min)

FIGURE 6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Relative Ion Abundance
100
Total Ion Current
0
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15
25
35
45
Time (min)

FIGURE 7

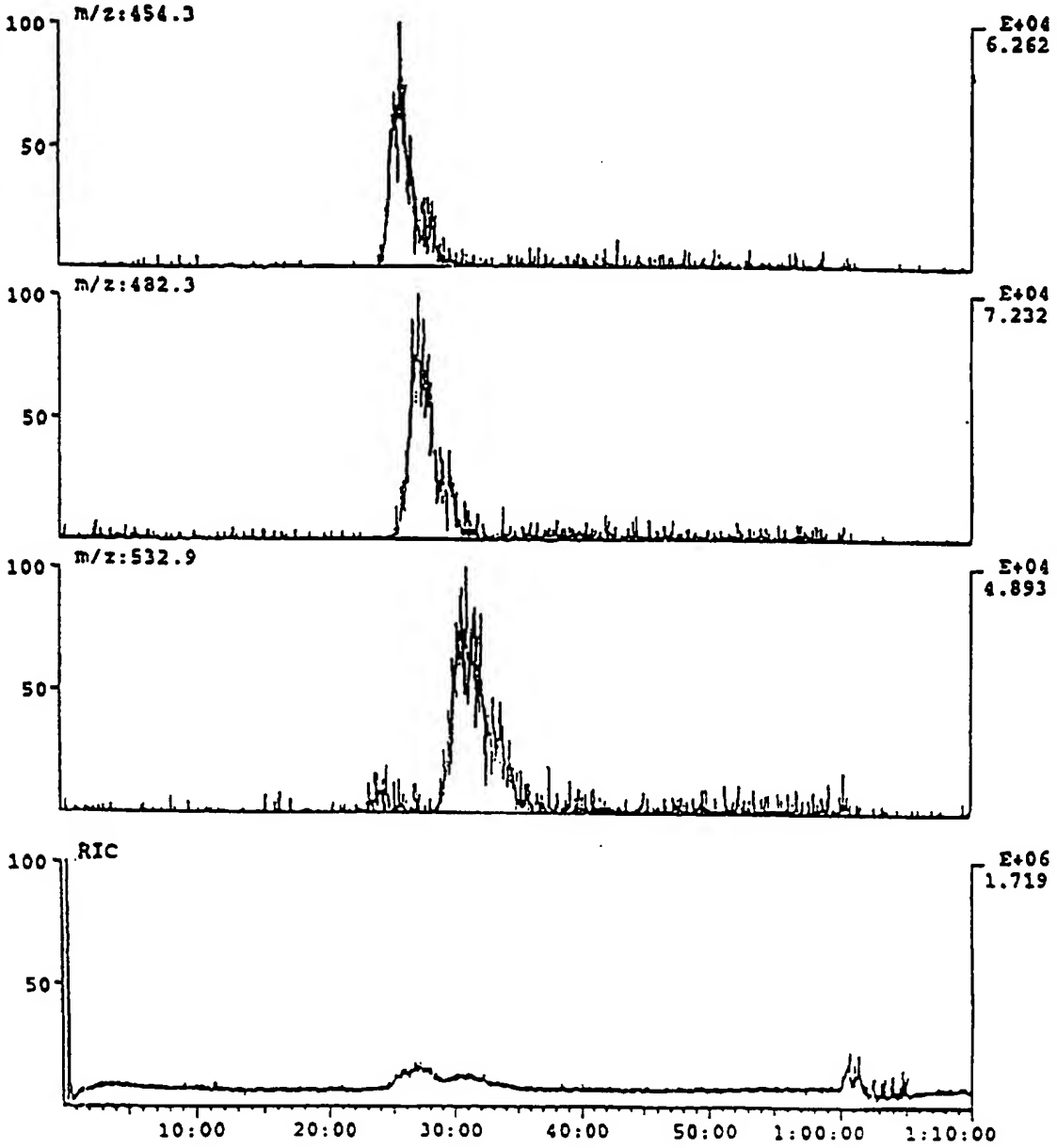
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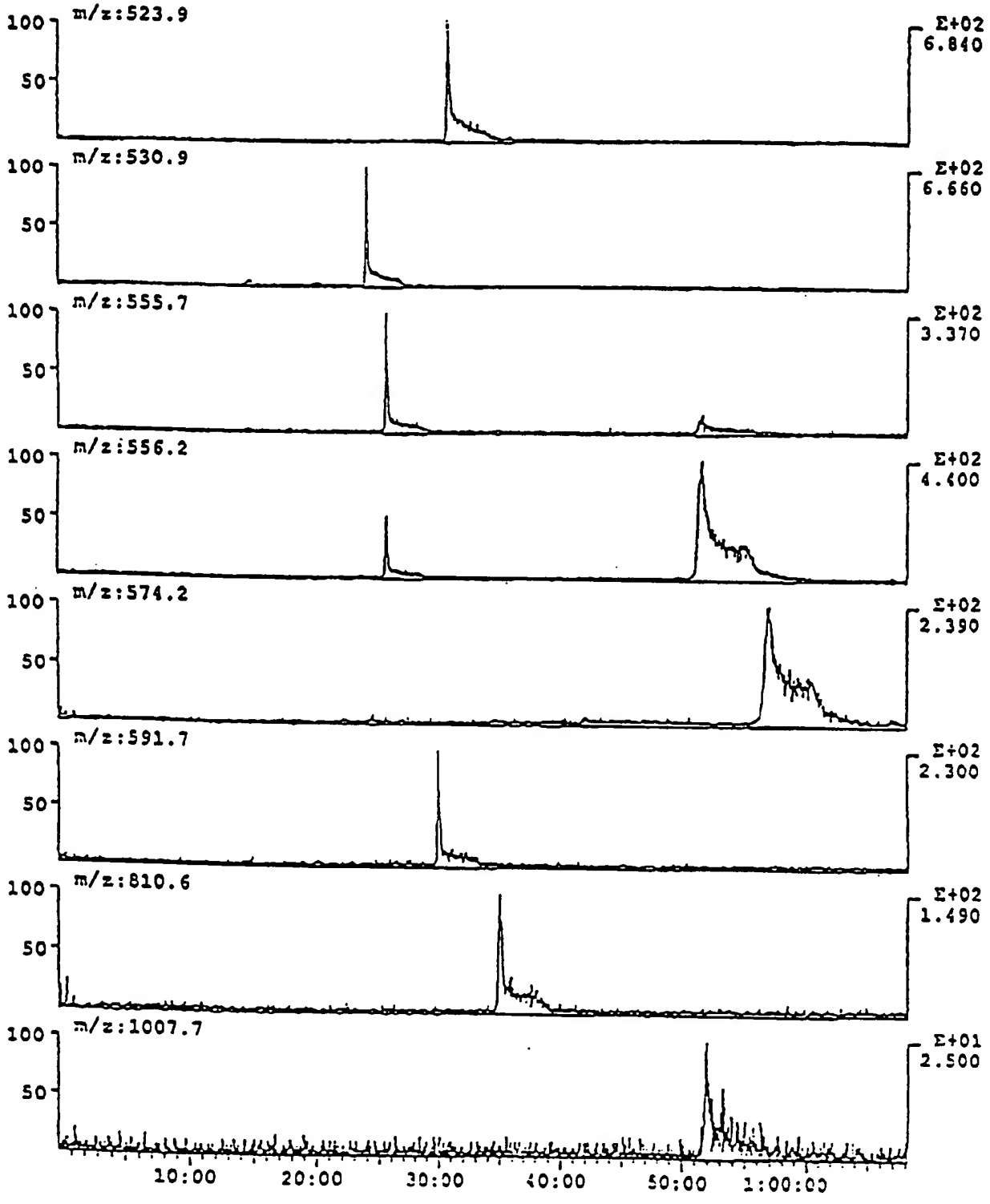
FIGURE 8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2.390
m/z:591.7
Σ+02
2.300
m/z:810.6
Σ+02
1.490
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Σ+01
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100
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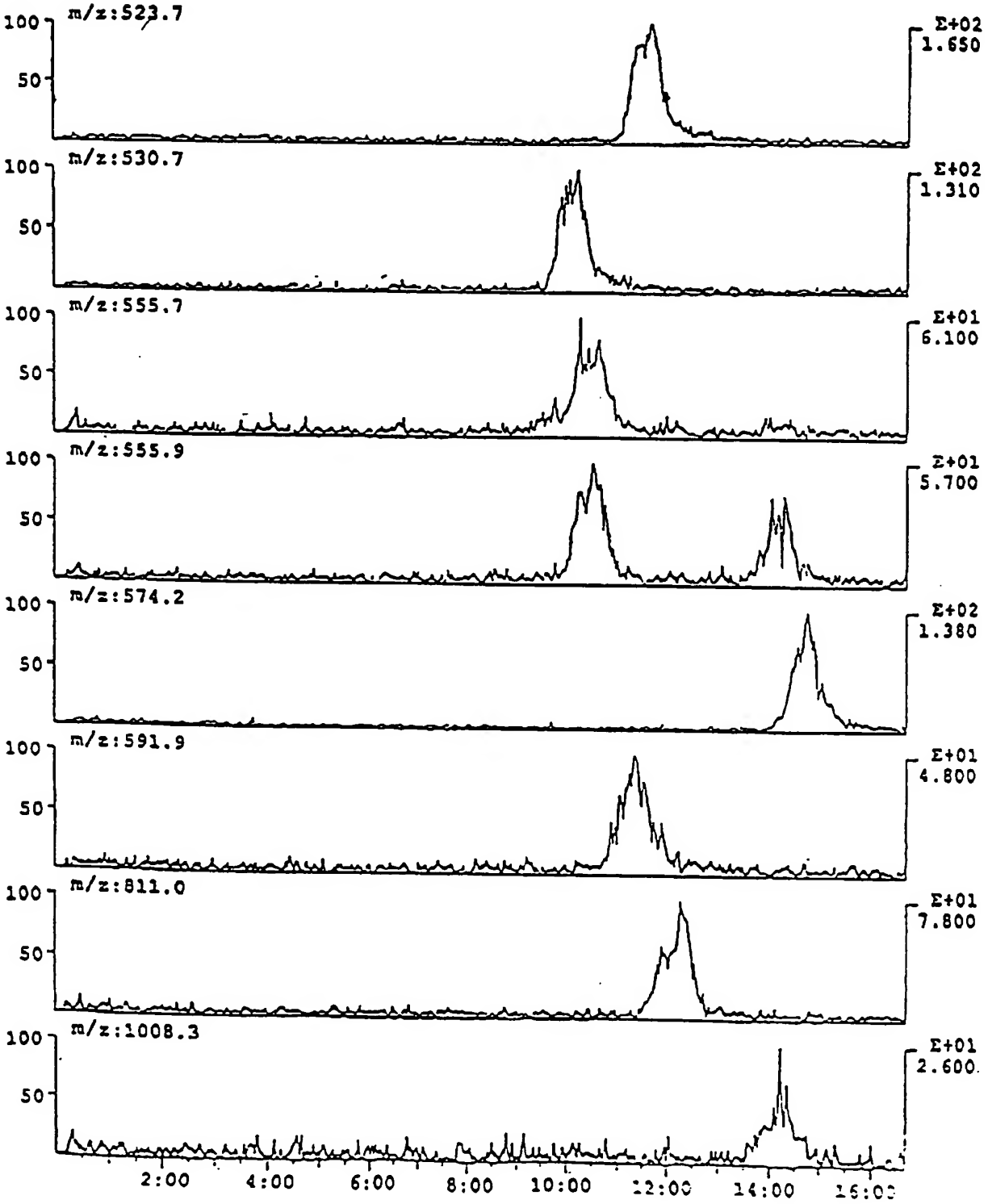
10:00
20:00
30:00
40:00
50:00
1:00:00

FIGURE 9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Σ+02
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Σ+01
6.100
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5.700
m/z:574.2
Σ+02
1.380
m/z:591.9
Σ+01
4.800
m/z:811.0
Σ+01
7.800
m/z:1008.3
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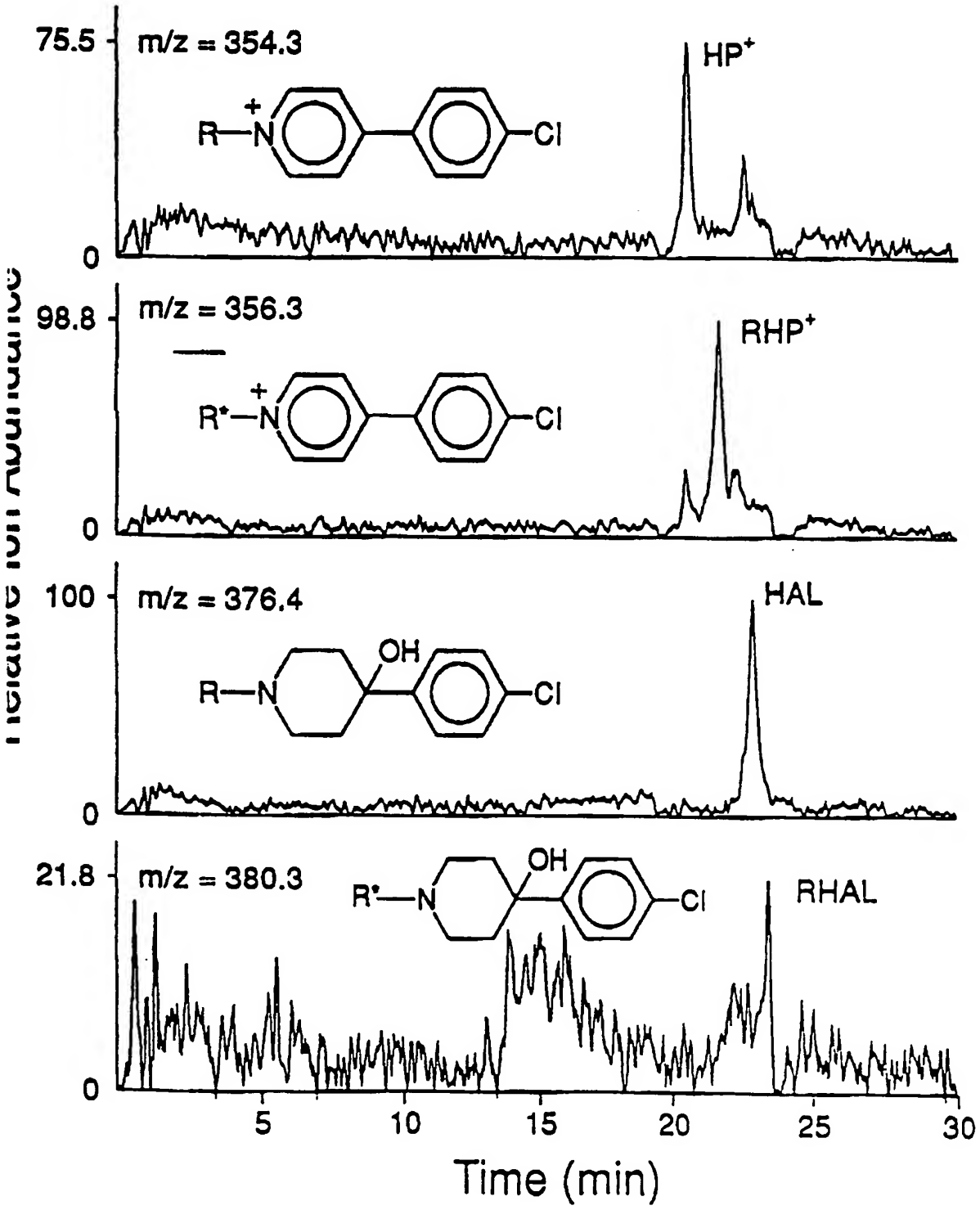
10:00
12:00
14:00
16:00

FIGURE 10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R—N+
Cl
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m/z = 356.3
RHP+
R*—N+
Cl
0
100
m/z = 376.4
HAL
OH
R—N
Cl
0
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m/z = 380.3
OH
R*—N
Cl
RHAL
0
5
10
15
20
25
30
Time (min)

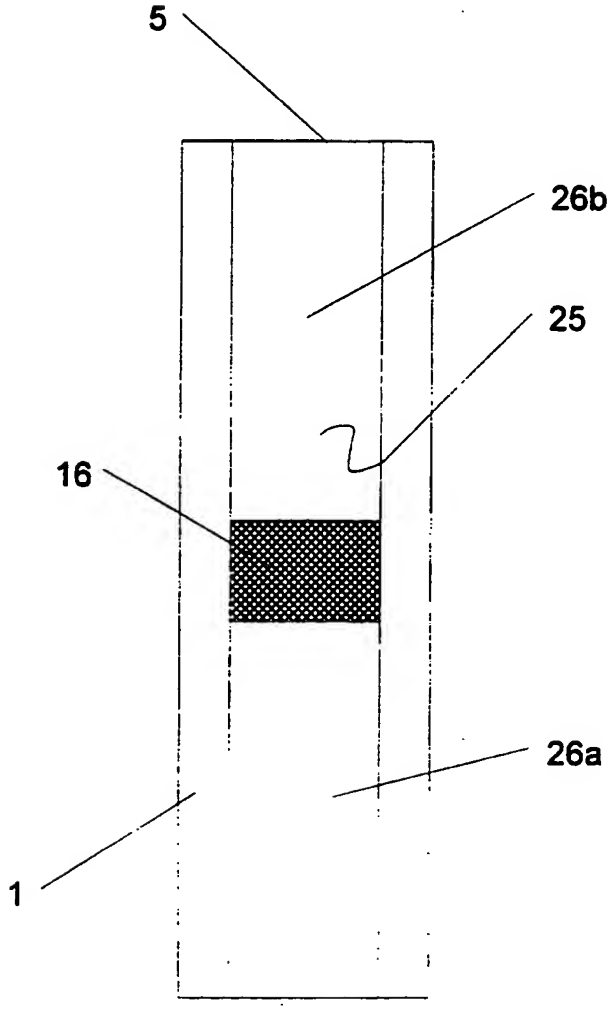


Figure 11

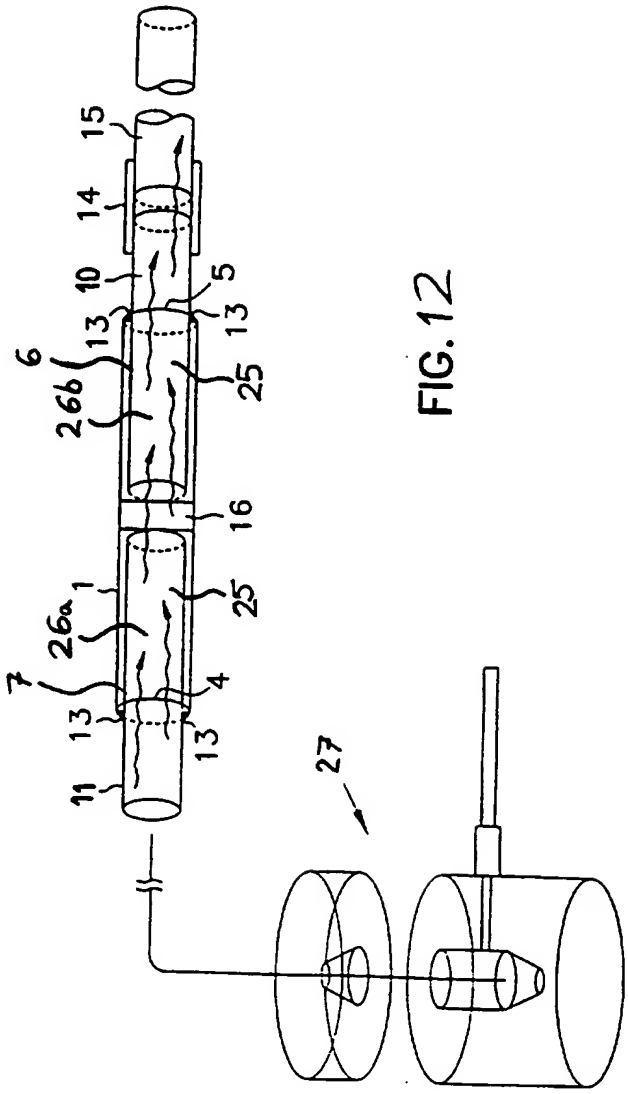


FIG. 12

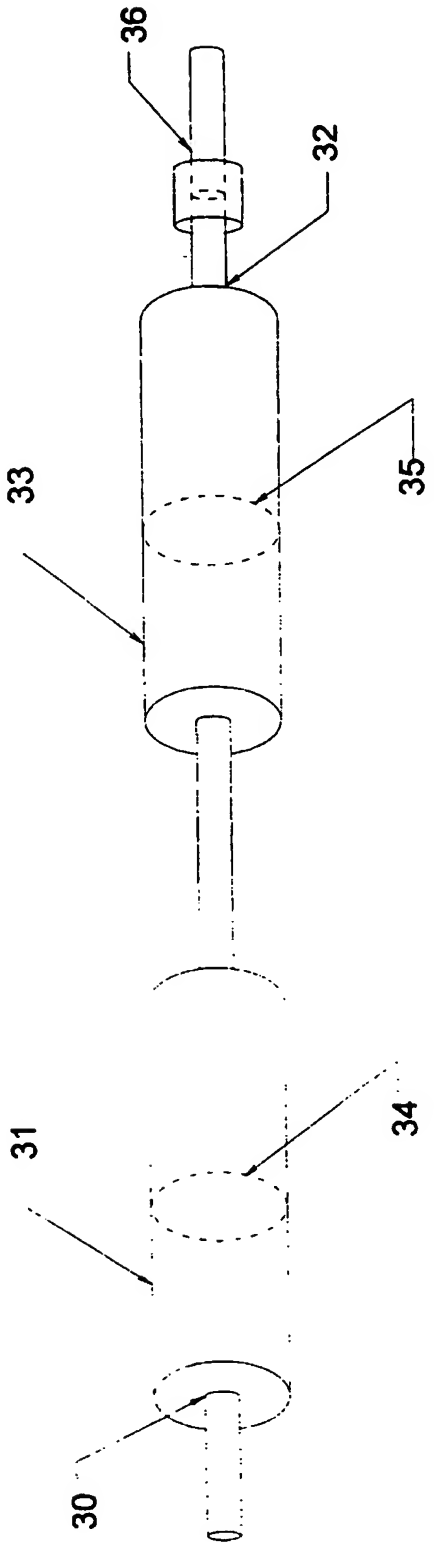
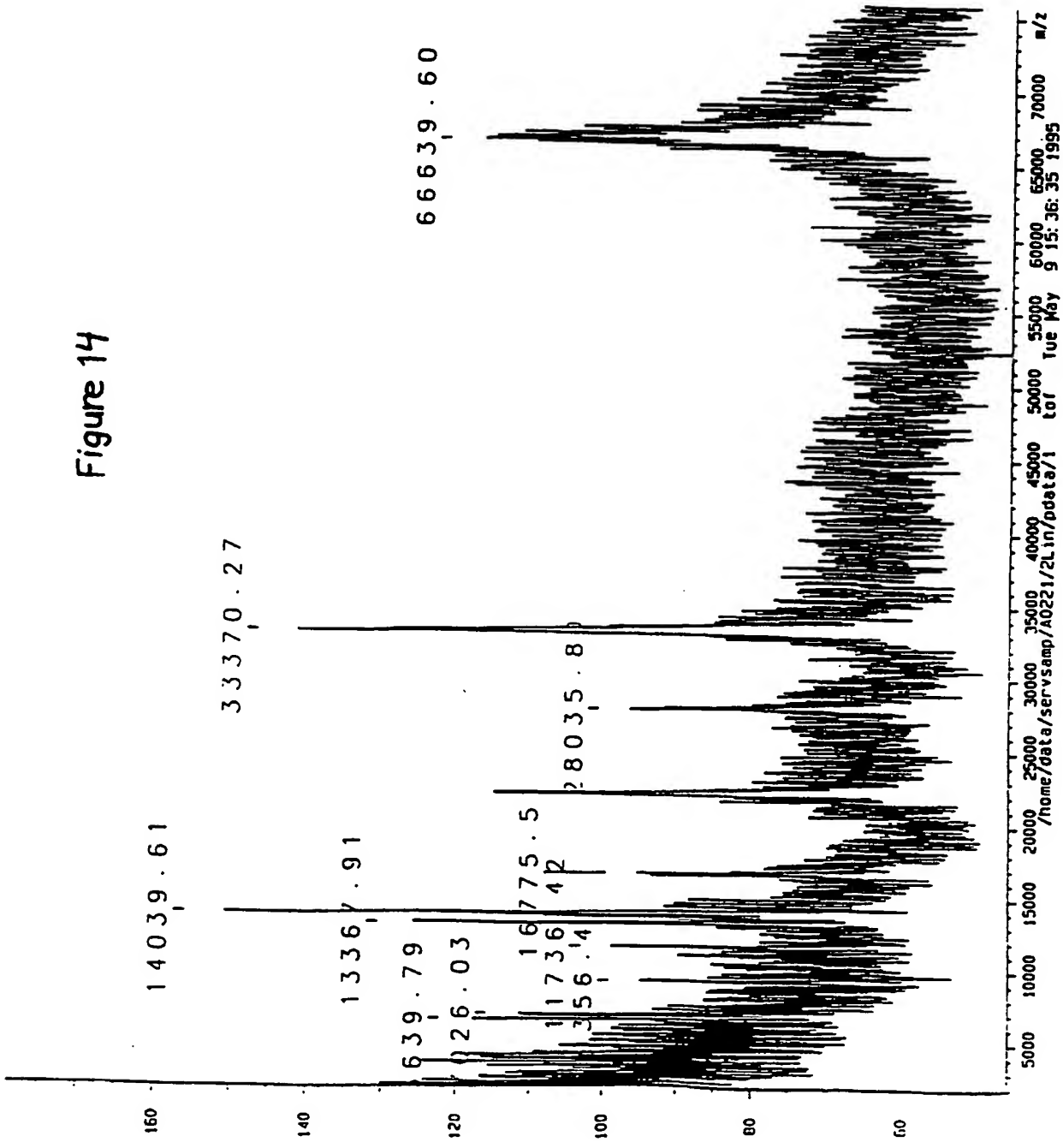


Figure 13

Figure 14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tof
Tue May 9 15:36:35 1995

INTERNATIONAL SEARCH REPORT

Interr ual Application No
PCT/US 96/05307

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N27/447

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 459 241 (MILLIPORE CORP.) 4 December 1991 see abstract; figures 1-4 ---	1
Y	JOURNAL OF HIGH RESOLUTION CHROMATOGRAPHY, vol. 17, no. 10, October 1994, pages 729-731, XP002007449 A. J. TOMLINSON: "ON-LINE PRECONCENTRATION-CAPILLARY ELECTROPHORESIS-MASS SPECTROPMETRY (PC-CE-MS)" see the whole document --- -/--	1

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
4 July 1996	30.07.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Duchatellier, M

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INTERNATIONAL SEARCH REPORT

Inter ·ial Application No
PCT/US 96/05307

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF CHROMATOGRAPHY, vol. 632, 1993, AMSTERDAM, NL, pages 209-213, XP002007450 M. E. SWARTZ: "ON-LINE SAMPLE PRECONCENTRATION ON A PACKED-INLET CAPILLARY FOR IMPROVING THE SENSITIVITY OF CAPILLARY ELECTROPHORETIC ANALYSIS OF PHARMACEUTICALS" see the whole document	1
A	WO,A,93 05390 (N. GUZMAN) 18 March 1993 see abstract; figure 31	1
A	EP,A,0 500 211 (BECKMAN INSTRUMENTS, INC.) 26 August 1992 see abstract; figure 1	1
A	EP,A,0 471 949 (HEWLETT-PACKARD CO.) 26 February 1992 see abstract; figure 2	1
A	ANALYTICAL CHEMISTRY, vol. 66, no. 21, November 1994, WASHINGTON, DC, US, pages 3696-3701, XP000483806 L. FANG: "ON-LINE TIME-OF-FLIGHT MASS SPECTROMETRIC ANALYSIS OF PEPTIDES SEPARATED BY CAPILLARY ELECTROPHORESIS" see the whole document	1
A	US,A,4 705 616 (B. D. ANDRESEN) 10 November 1987 see abstract; figure 1	1
A	EP,A,0 297 149 (HEWLETT-PACKARD GMBH) 4 January 1989 see abstract; figure 1	1

1

INTERNATIONAL SEARCH REPORT

information on patent family members

Inter ...al Application No
PCT/US 96/05307

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		CA-A-	2120251	18-03-93
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